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EFFECT ON MONKEYS OF SMALL DOSES OF A CONCENTRATED PREPARATION OF VIOSTEROL

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It was with the idea that repeated dosage with viosterol may really be harmful, despite complete absence of clinical signs of injury, that these experiments were undertaken. Since the tissues of children fed viosterol are obviously not available for examination except in rare cases of sudden death from some cause having nothing to do with the treatment, we decided to make use of young monkeys (Macacus rhesus). But this unavoidable shift from man to an experimental animal complicated matters. In order to measure the effect of the drug, we had first to establish the normal range in variation for all the tissues studied in this species, and having ascertained the changes we were faced by the much more difficult problem of reaching a decision as to how far we might expect similar effects in children.

The only data we have been able to find in the literature on the response of monkeys to viosterol are contained in two brief notes by Hess and his associates. In the first [1] it was reported that large amounts of viosterol given by mouth promptly raised the serum calcium to a normal level in a monkey in which latent tetany had been induced and maintained for several months by a diet low in calcium. In the second [2] the results of "numerous extirpation experiments on monkeys and dogs" were described, but the number of animals of each species employed was not stated. The authors observed that hypercalcemia amounting to from 13 to 16 mg. per hundred cubic centimeters of serum could be induced before operation, but that after extirpation it was not possible to raise the calcium above normal.

In an extensive survey Fox [3] found that arterial disease is not common in monkeys. He examined seven hundred and ninety-six animals, that is to say,

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1. Hess, A. F., and Lewis, J. M.: J. A. M. A. **91**:783, 1928.

2. Hess, A. F.; Weinstock, M., and Rivkin, H.: Proc. Soc. Exper. Biol. & Med. **26**:555, 1929.

3. Fox, Herbert: Arteriosclerosis in Lower Mammals and Birds: Its Relation to the Disease in Man, in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, p. 153.

mammals extending from the highest apes to and including the marmosets, and found only sixteen cases of arteriosclerosis. But he called attention to the fact that his series did not include monkeys of the species Macacus rhesus; nor have we been able to discover any account of arterial disease in Macacus rhesus. If, in this respect, Macacus rhesus resembles other species of monkeys, it is likely that we are dealing with animals in which spontaneous arterial lesions are uncommon as contrasted with rabbits, for example, in which medial sclerosis has been frequently reported to be present. This is important because it suggests that any arterial lesions not observed in our controls but present in the treated animals were probably caused in some way by the treatment and are not likely to have been of spontaneous origin.

MATERIAL

A concentrated preparation of viosterol containing 920,000 U.S.P. XI units of vitamin D per cubic centimeter, or approximately 1,000,000 units per gram,[4] was employed for all monkeys except monkey 137, which received viosterol of the usual potency (10,000 U.S.P. XI units of vitamin D per gram[5]).

In order to test the activity of the particular sample of the concentrated preparation of viosterol employed twelve rats were given doses equivalent, as proportioned to weight, to those administered to monkey 14. Their blood calcium increased, some died, and the reaction in general closely resembled that described in detail by Ham.[6]

The weights of the nineteen treated monkeys (numbered 1 to 19) ranged from 2,530 to 3,270 Gm., with an average of 2,783.2 Gm. Their exact age is unknown, but their dentition was complete and the cranial sutures were united. The gonads of eleven of the nineteen monkeys were examined histologically. Five of these were males and exhibited no spermatogenesis, whereas the remaining six females all possessed ripe follicles. The living conditions before and during the experiments were good. The animals were allowed to stay indoors or outside in the sun or shade as they wished, and there was plenty of room for exercise.

The twenty-four untreated monkeys used as controls (designated by the letters from A to X) were a less homogeneous series. Their weights varied from 2,453 to 8,745 Gm., with an average of 3,904.6 Gm., which was high because of the inclusion of four rather heavy animals. The testes of seven showed active spermatogenesis while those of three were inactive. The ovaries of six contained ripe follicles. Seven were normal and strictly comparable with the experimental animals. These were killed from two weeks to two months after receipt

4. This preparation corresponds to the one formerly designated as viosterol 10,000 X, which was used by C. I. Reed (J. A. M. A. **102**:1745, 1934). It was supplied by Mead Johnson & Co.

5. This is the standard preparation, described in the "Pharmacopoiea of the United States" (XI) as solution of irradiated ergosterol and in "New and Nonofficial Remedies" as viosterol. It also was manufactured by Mead Johnson & Co.

6. Ham, A. W.: Arch. Path. **14**:613, 1932.

from the dealer (A to G). Eight had been inoculated with material from patients with trachoma, but the temporary reaction had subsided and there was no reason to suppose that any general systemic altera-

TABLE 1.—*Summary of Experimental Data* *

Monkey	Sex	Weight, Gm.	Range of Blood Calcium and Phosphorus Before Treatment, Gm.	Kind of Treatment	Range of Blood Calcium and Phosphorus During Treatment, Gm.
1	F	3,270	Ca 9.64 - 10.8 (4) P 7.56 - 8.57 (3) 3 days	30 cc. (3) 30 days	Ca 10.65 - 12.67 (5) P 7.06 - 8.57 (5)
2	M	2,890	Ca 10.8 - 12.94 (4) P 7.4 - 7.80 (4) 60 days	12.5 cc. (2) 6 days	Ca 10.8 - 12.94 (2) P 7.5 - 7.5 (2)
4	F	2,730	Ca 10.77 - 11.53 (6) P 5.72 - 6.10 (7) 126 days	29 cc. (27) 147 days	Ca 9.76 - 17.10 (21) P 4.6 - 6.9 (22)
5	F	2,950	Ca 10.0 - 11.75 (8) P 4.91 - 6.32 (10) 124 days	10.5 cc. (19) 133 days	Ca 9.11 - 10.8 (17) P 5.12 - 10.9 (17)
6	M	3,800	Ca 11.6 - 13.2 (7) P ..... 14 days	5 cc. (1) 4 days	Ca 12.0 - 13.8 (4) P .....
7	M	2,880	Ca 10.5 - 12.12 (8) P 5.37 - 6.70 (6) 127 days	36.5 cc. (26) 133 days	Ca 9.47 - 11.5 (22) P 5.97 - 12.5 (21)
8	M	2,530	Ca 11.13 - 11.60 (8) P ..... 30 days	10 cc. (2) 3 days	Ca 11.08 - 12.05 (5) P .....
9	M	2,530	Ca 10.1 - 12.0 (10) P 5.11 - 5.83 (7) 130 days	14 cc. (22) 134 days	Ca 10.2 - 12.16 (21) P 5.0 - 9.33 (20)
10	F	2,560	Ca 10.7 - 11.36 (11) P 5.01 - 5.85 (6) 130 days	30 cc. (27) 134 days	Ca 9.7 - 20.3 (21) P 5.2 - 9.38 (20)
11	F	2,890	Ca 10.5 - 11.0 (2) P 4.72 - 7.41 (11) 48 days	33 cc. (28) 134 days	Ca 9.87 - 12.81 (21) P 5.5 - 9.73 (21)
13	F	3,800	Ca 11.2 - 14.0 (11) P ..... 24 days	10 cc. (2) 35 days	Ca 11.2 - 12.1 (6) P .....
14	M	2,830	Ca 11.27 - 12.44 (7) P 5.82 - 6.32 (8) 124 days	125 cc. (110) 98 days	Ca 10.1 - 17.1 (19) P 5.15 - 8.82 (18)
15	M	3,800	Ca 11.5 - 14.8 (10) P ..... 19 days	5 cc. (1) 4 days	Ca 11.1 - 11.8 (4) P .....
16	F	2,640	Ca 10.0 - 10.1 (2) P 4.47 - 6.21 (10) 8 days	6.5 cc. (13) 91 days	Ca 10.2 - 10.6 (9) P 6.69 - 10.5 (9)
17	M	2,820	Ca 10.77 - 11.42 (9) P 5.14 - 6.30 (9) 90 days	13 cc. (20) 134 days	Ca 6.55 - 11.6 (19) P 4.89 - 9.87 (19)
18	M	2,950	Ca 10.5 - 11.57 (9) P 5.66 - 6.31 (7) 120 days	133 cc. (28) 134 days	Ca 9.74 - 18.4 (23) P 4.8 - 9.75 (23)
19	F	2,870	Ca 9.8 - 10.2 (2) P 4.43 - 6.32 (11) 55 days	13 cc. (20) 134 days	Ca 9.96 - 12.5 (19) P 5.0 - 7.63 (17)

* See text, p. 4 and 5 for full explanation of the matter in the columns.

tions had occurred which would result in changes like those to be expected from treatment with the two preparations of viosterol. The time after purchase was longer, being from six months to one year (H to O). One died of tuberculosis (P). Three were killed in severe

experimental poliomyelitis (Q, R and S). One was killed after it had recovered from a mild attack of experimental poliomyelitis (T). One large male (given to us by Dr. M. G. Seelig) was inoculated with syphilis and kept in the laboratory of the Bernard Free Skin and Cancer Hospital for fifteen years. It was in excellent physical condition when killed (U). Two additional monkeys from the Trachoma Commission of Washington University, obtained through Dr. L. A. Julianelle, were listed as W and X.

As to diet, monkeys 1, 2, 6, 8, 13 and 15 were given 82 Gm. of solids, 190 Gm. of tomato pulp and juice and water ad libitum per day. The solids were:

	Gm.	Per Cent
Skim milk powder	800	24
Sugar	400	12
Salt	35	1
Cornstarch	525	16
Oats	800	23
Yellow corn	400	12
Egg yolks	400	12
Total	3,360	100

This gave 10 per cent protein, 65 per cent carbohydrate and 3 per cent salts.

All of the remaining untreated and treated monkeys were allowed as much Purina dog chow,[7] tomato pulp with juice and water as they wished. The ratio of calcium to phosphorus was 1.9:1, and the vitamins were adequate.

CHEMICAL CHANGES

Our five main experiments were performed at various times during the past three years in order to ascertain the effect of: (1) single large doses (monkeys 6 and 15); (2) double doses (monkeys 8 and 13); (3) double doses preceded or not preceded by a single one (monkeys 1 and 2); (4) repeated doses over long periods (monkeys 5, 7, 9, 11, 16, 17 and 19); (5) doses heavy enough to increase the blood calcium (monkeys 4, 14 and 18).

The chemical analyses were made by Dr. Max Möller and Dr. D. J. Kooyman.

For calcium the method of Kramer and Tisdall was employed, and for phosphorus, that of Collip as modified by Bulger. For the sake of brevity the results are presented collectively in table 1 instead of under five headings. There are six columns. In the first three columns the number, sex and weight of the monkey are given; in the fourth, the range (minimum and maximum) in serum calcium and phosphorus

7. The Purina Mills Company supplied the following analysis of the chow: 5 per cent fat, 23 per cent protein, 4 per cent fiber, 7 per cent ash, 7 per cent moisture and 54 per cent nitrogen-free extract.

before treatment together with the number of determinations (in brackets) made during the time stated; in the fifth, the total volume of the concentrated preparation of viosterol, with the number of individual doses (in brackets) and the time between the first dose and the time when the animal was put to death for collecting histologic material; in the sixth, the range in calcium and phosphorus with the number of determinations (in brackets) during the period of treatment. The graphs for each animal are available to any person interested.

Calculations from data available but not shown in table 1 yielded the ranges listed in table 2. The average amount of serum calcium for the normal males is 0.63 mg. higher than that for the normal females. The range for all observations of serum calcium in the males before treatment is from 10 to 14.8 mg., and the range in any one monkey of the series is from 11.5 to 14.8 mg. For the females these amounts are slightly but consistently lower, from 9.64 to 14 and from 11.2 to

TABLE 2.—*Averages and Ranges of Serum Calcium and Phosphorus*

Point of Comparison	Before Treatment, Gm.	During Treatment, Gm.
Average serum Ca for males	11.62	11.32
Average serum Ca for females	10.99	10.98
Average serum P for males	5.98	7.75
Average serum P for females	6.02	7.44
Range serum Ca for males, lowest and highest level in series	10.0 - 14.8	6.55 - 18.4
Range serum Ca for females	9.64 - 14.0	9.11 - 20.3
Range serum P for males	5.11 - 7.8	4.8 - 12.5
Range serum P for females	4.43 - 8.57	4.6 - 10.9
Maximum range Ca for males	11.5 - 14.8	9.74 - 18.4
Maximum range Ca for females	11.2 - 14.0	9.70 - 20.3
Maximum range P for males	5.37 - 6.70	5.97 - 12.5
Maximum range P for females	4.72 - 7.41	5.12 - 10.9

14 mg., respectively. It seems likely, judging from these rather sharply delimited ranges, that the normal limits of variation in monkeys under the conditions described are of the order of 2.5 mg. or slightly more above the averages given and rather less than that below. The data from which these averages and ranges were derived show that in the majority of cases the deviations from the mean given are very apt to be decidedly less.

Determinations of serum phosphorus in the same series of animals are interesting in that the percentage deviations are somewhat greater than those for serum calcium. The average amount of serum phosphorus for the males is not significantly different from that for the females, but the difference between the total ranges is greater by more than twofold, the greatest scatter being found in the figures for the females. The same is true of the largest individual range. Study of the data tends to confirm the impression that both collectively and individually the female monkeys consistently showed a greater normal variation in serum phosphorus than did the males.

During treatment with the concentrated preparation of viosterol the males were subject to greater variation in serum calcium than the females. The average amount of serum calcium for the treated males is 11.32 mg. as against 11.62 mg. for the normal ones. The lowering of the mean value by 0.3 mg. is not significant nor, when one considers that the serum calcium in two of the nine males ran up to 17.1 and to 18.4 mg., does the average cease to represent a true value, as in these cases the higher figures are at most but three of nineteen and twenty-three observations, respectively. The mean (10.98) given for females is subject to the same comment in view of the fact that two of the eight animals had serum calcium as high as 17.1 and 20.3 mg. per hundred cubic centimeters, but these represent only three of twenty-one determinations, respectively. The total range for males undergoing treatment is from 6.55 to 18.4 mg., or a spread of 11.85 mg., while that for females is from 9.11 to 20.3 mg., a scatter of 10.19 mg. The greatest individual range for males as shown in table 2 is from 9.74 to 18.4 mg., while that for females is from 9.7 to 20.3 mg.

The average amount of serum phosphorus for treated animals, both males and females, is raised in about the same order of magnitude. The mean value for treated males is slightly but not significantly higher than that for females. However, the total range for males is somewhat greater than that for females. This is true also for the greatest individual range.

The chemical findings before and during treatment indicate clearly that small repeated doses of the concentrated preparation of viosterol did not alter appreciably the average amount of serum calcium but increased by about 25 per cent the average amount of serum phosphorus. They indicate further that it is possible by repeated massive doses and even single doses to increase the serum calcium to a marked degree. From our observations it seems logical to conclude that in general males are somewhat more capricious in their response to the treatment than females.

To give point to the histologic account the treated animals are listed in three categories: (1) those whose serum calcium remained within the maximum range before treatment (monkeys 1 to 3, 5 to 9, 11 to 13, 15 to 17, 19 and 20); (2) those whose serum calcium was increased without clinical signs of injury (monkeys 4, 10, 14 and 18); (3) those whose serum calcium was increased with evidence of injury (monkeys 4 and 14).

HISTOLOGIC CHANGES

Complete autopsies were made on all treated and control monkeys. Tissues were fixed in Zenker's fluid without acetic acid plus diluted solution of formaldehyde U.S.P. (1 : 10) or in absolute alcohol, 9 parts,

and solution of formaldehyde, 1 part. Carefully oriented transverse sections or arteries fixed in the first way were colored with hematoxylin and eosin as a routine, and for special purposes, with Mallory's connective tissue stain and resorcinol fuchsin. Similar sections of arteries preserved in the second way were incinerated by the method of Scott.[8] Hematoxylin and eosin preparations were made of the principal organs as a routine. In addition, sections of the kidneys were colored by Mallory's method and with resorcinol fuchsin. Since the likelihood of detecting histologic modifications depends on the scope of the examination, the following list of tissues is submitted:

Tissue	Treated Monkeys	Controls
Aortic arch, thoracic aorta, abdominal aorta, right subclavian artery, right common carotid artery, internal carotid artery, and anterior cerebral and middle cerebral arteries	18	21
Common iliac and femoral arteries	18	20
Innominate, right brachial and left radial arteries	17	21
Pulmonary and central retinal arteries	17	20
Basilar artery	18	19
Colon, ileum, pancreas, skeletal muscle and submaxillary gland	10	16
Adrenal gland and lung	12	16
Cerebrum and cerebellum	3	14
Bone	2	10
Bone marrow	3	13
Duodenum	4	14
Esophagus and jejunum	8	14
Heart	19	20
Kidney	16	16
Liver	19	16
Lymph node	9	15
Mucous membrane of mouth	5	16
Ovary	6	5
Parathyroid gland	7	14
Parotid gland	8	15
Pituitary gland	5	9
Prostate gland	4	2
Skin	4	13
Spleen	12	14
Stomach	13	16
Testis	5	9
Thymus	9	13
Thyroid	11	16
Trachea	10	15
Urinary bladder	11	14
Uterus	5	5
Vagina	3	2

8. Scott, Gordon H.: Protoplasma **20**:133, 1933.

Some of the alterations observed are listed in table 3. By reference also to table 1 and to the brief description of the monkeys employed the conditions in any one may be established. We shall compare the group of treated monkeys with the group of controls to avoid the repetition that would be necessary if each were described separately, particularly in experiments like ours designed to reveal the elusive and much neglected influence of small doses.

Our major contribution concerns arteries, parathyroid glands and kidneys, which will therefore be described in some detail. Following this, short accounts will be presented of modifications in other tissues

TABLE 3.—*Incidence of Some Lesions in Untreated and Treated Animals*

Lesion	Untreated	Treated
Calcification and fragmentation of internal elastic membrane	None of 21	1 (14) of 16
Thickening of intima with basophilia	1 (F) of 21	8 (3, 7, 8, 9, 12, 14, 15, 19) of 18 (fig. 1A)
Thickening of intima with rarefaction	4 (E, F, G, T) of 21	8 (3, 7, 9, 10, 12, 15, 17, 18) of 18 (fig. 1B)
Basophil cytoplasmic bodies in parathyroid glands	Marked in 4 (G, N, M, V), easily visible in 7 (E, F, H, I, J, K, L), detectable in 3 (A, C, D), absent in none of 14 (fig. 2B)	Marked in none, easily visible in 2 (2, 7), detectable in 3 (5, 10, 11), absent in 2 (1, 4) of 7 (fig. 2A)
Renal interstitial tissue fibrosis	1 (H) of 16	3 (4, 10, 14) of 16
Renal interstitial lymphocytic infiltration	5 (H, J, N, U, V) of 16	7 (1, 4, 10, 14, 16, 18, 19) of 16
Thickening of epithelium of Bowman's capsule	8 (A, E-I, L, U) of 16	7 (4, 5, 6, 7, 10, 15, 19) of 16
Glomerulonephritis	None of 16	2 (10, 19) of 16 (fig. 3)
Inclusions in nuclei of renal tubule cells	2 (A, N, V) of 16	12 (3, 4, 5, 6, 7, 10, 11, 13, 14, 16, 18, 19) of 16
Renal tubular necrosis.....	None of 16	2 (11, 14) of 16 (figs. 4B and 5B)
Tubular regeneration	None of 16	2 (4, 5) of 16
Hyaline casts in renal tubules	Frequent in 2 (C, U) of 16	Rare in 2 (10, 19) of 16
Leukocytic invasion of renal tubules	None of 16	3 (4, 10, 18) of 16 (fig. 5A)

and of supplementary experiments which cannot be altogether omitted if a comprehensive view of the experimental animals is to be presented.

The only arterial modifications of which no trace was seen in the controls were calcification and fragmentation of the internal elastic membrane of the left radial artery, seen in monkey 14.

Thickening of the intima, with basophilia and increase in mineral matter shown by micro-incineration, was much more frequent in the treated than in the control animals. The maximum noted was in the abdominal aorta of monkey 8 (fig. 1*A*). The distribution was: thoracic aorta (17); abdominal aorta (3, 7, 8, 9, 12, 14, 15 and 19); common iliac artery (3, 8, 14, 15 and 19); right subclavian artery (3); right brachial artery (9). Among the controls only one exhibited a similar

alteration (or deviation from the average), located in the innominate, right subclavian and internal carotid arteries.

Thickening of the intima with rarefaction but no basophilia was likewise more frequent in the treated monkeys. The maximum change was in the common iliac artery of monkey 18 (fig. 1*B*). The distribution was: thoracic aorta (17); abdominal aorta (3, 7, 9, 10, 12, 15, 17 and 18); common iliac (10, 18 and 19). In the controls it was limited to the abdominal aortas of five monkeys. We did not employ sudan III, but the few cells in rarefied areas looked as if they might be rich in fat and lipoid. Our figure 1*B* has some points of resemblance to figures published by Nuzum and others [9] illustrating spontaneous "intimal arteriosclerosis" in rabbits. The thickenings were not identified during

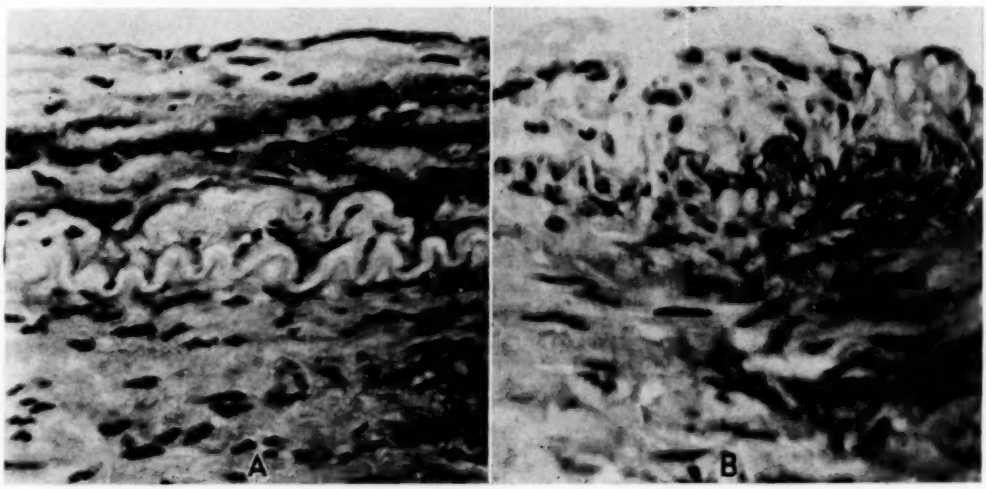

Fig. 1.—*A*, abdominal aorta of treated monkey 8, illustrating maximum thickening of intima with basophilia and increase in mineral constituents; *B*, common iliac artery of treated monkey 18, showing maximum thickening of intima with rarefaction and without basophilia, mineralization or stratification; × 450.

the gross inspection of the large vessels split longitudinally which was made in each autopsy. Neither did we see "superficial fatty streaks" comparable to those in normal human aortas described by numerous authors.[10] Incidentally, it may be mentioned that smooth muscle occurs sparsely in the aortic intima of Macacus rhesus—apropos of the much discussed question whether such muscle occurs in the human aortic intima.[11]

9. Nuzum, F. R.; Elliott, A. H.; Evans, R. D., and Priest, B. V.: Arch. Path. **10**:697 1930.

10. McMeans, J. W., and Klotz, O.: J. M. Research **34**:41, 1916. Lange, F.: Virchows Arch. f. path. Anat. **248**:463, 1924.

11. Ollendorff, A.: Anat. Anz. **38**:569, 1911.

We looked particularly for traces of medial calcification and discovered in the pulmonary artery of control monkey G a basophilic band a little less marked than that represented by Nicole [12] in his figure 4; but no comparable alteration was detected in any of the treated monkeys. Muscular changes such as those reported by Wenzel [13] were not seen.

It is evident, therefore, that treatment with the concentrated preparation of viosterol produced alterations (or intensifications of normally occurring conditions) in the intima of the elastic arteries. Except for the change in the radial artery, the muscular arteries exhibited no modifications when examined by the technic mentioned. To our surprise the arterioles seemed normal.

Table 3 indicates that certain basophilic bodies in the parathyroid glands were less prominent in the treated than in the control animals (compare fig. 2*A* and *B*). These bodies are normally most noticeable in slightly elongated cells, in which they are prone to occur at one or both poles of the nucleus, separated from the nucleus, however, by pale-staining cytoplasm. No information as to their nature is to be found in the literature, but they have undoubtedly been seen by many investigators before us, for they are conspicuous features of most parathyroid glands employed for routine teaching in histology. The "juxtanuclear" body mentioned by Pappenheimer and Wilens [14] probably belongs in this category.

In order to ascertain whether a change in the size of the cells of these glands took place, the nuclei of the epithelial cells were counted:

We inserted in our microscopic ocular a disk on which a ruled square was subdivided by vertical and horizontal lines into 100 smaller squares. We calibrated this so that with a given objective and ocular the large square covered an area of the section to be examined which was 366 by 366 microns. Our counts were restricted to parathyroid glands partly or completely surrounded by thyroid tissue and fixed in Zenker's fluid plus a diluted solution of formaldehyde U. S. P. (1:10). The sections were stained with hematoxylin and eosin, and their thickness was 5 microns. With the microtome employed variations in thickness could not have introduced a serious error; but, to be on the safe side, the counts of each specimen were made at a single focus. Our ability to do this depended on the excellence of our optical equipment. To render the counts as directly comparable as possible, the ruled square was always superposed on a section in such a way that one side of the square was parallel to and just within the capsule of the gland while the remainder extended inward over the section. We are not sure that the inclination of inward extension was always exactly at right angles to the surface of the gland. Those sections which cut only the surface might have given a different impression from others which reached directly into the

12. Nicole, R.: Ztschr. f. d. ges. exper. Med. **70**:193, 1930.
13. Wenzel, H.: Arch. f. exper. Path. u. Pharmakol. **137**:215, 1928.
14. Pappenheimer, A. M., and Wilens, S. L.: Am. J. Path. **11**:73, 1935.

gland because in most parathyroid glands of monkeys the external parts are more cellular than the more deeply lying ones and in the latter there is a greater tendency toward the formation of acini.

In the twelve controls examined in this way we found, per unit area, a maximum of 1,966 nuclei, a minimum of 1,282 and an average of 1,605.2. In the seven treated animals whose parathyroid glands were examined, the maximum was 1,866, the minimum 1,463 and the average 1,621.5. If a less crowded condition of nuclei is an expression of hyperplasia, as in chickens,[15] hyperplasia was certainly not marked in our treated monkeys. Since extremely few of the epithelial cells contained more than a single nucleus each, these counts of nuclei can be regarded as counts of epithelial cells.

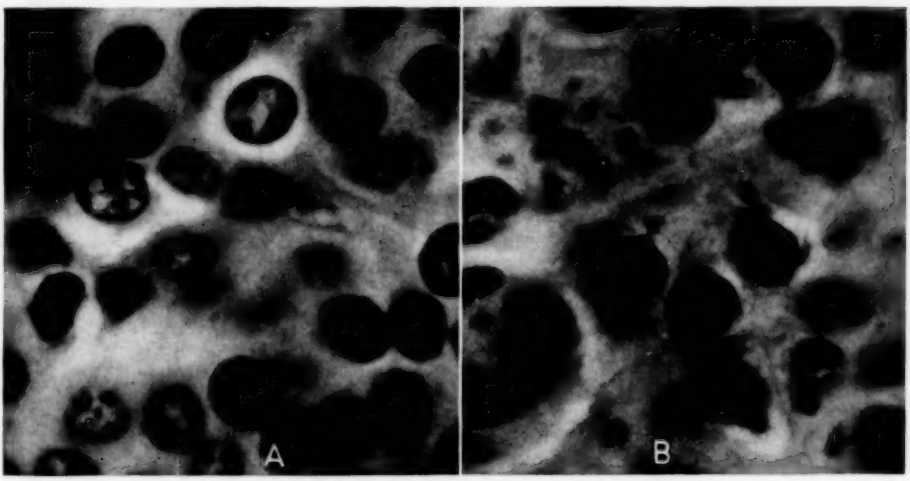

Fig. 2.—*A*, parathyroid gland of treated monkey 4, showing that basophilic masses have left the cytoplasm; *B*, parathyroid gland of control monkey G, exhibiting normal number of basophilic masses; × 1,700.

We have no information bearing on the presence or absence of enlargement of the parathyroid glands as a whole because, in collecting tissues, we did not isolate and weigh each of these glands. Instead, we simply chose pieces of thyroid gland that we thought included a parathyroid gland. Unfortunately we were mistaken in some cases, and this reduced the number of parathyroid glands from the treated animals below that of the controls. All the glands were of the compact type with only occasional fatty tissue and dilated lumens of acini which together would effect a reduction in the cellularity expressed by the counts. From the fact that, in general, the number of cells per unit

15. Nonidez, J. F., and Goodale, H. D.: Am. J. Anat. **38**:319, 1927.

area was about the same in the two series it follows that there was no great increase in the size of the cells in the treated animals. Therefore, cellular hypertrophy was not marked except as indicated by a slight but noticeable increase in the relative number of the larger clear and oxyphil cells, which we regard as derivatives of the chief cells. In making the counts only one mitotic figure was observed (in control animal H), so that unmistakable signs of hyperplasia were lacking. When search was extended to a larger area than that covered by the hundred small squares a few additional mitoses were found.

The chief alterations were in the kidneys, but they were not of the sort described by others before us, or were they expected. The slightly higher incidence of thickening of interstitial tissue and of lymphocytic

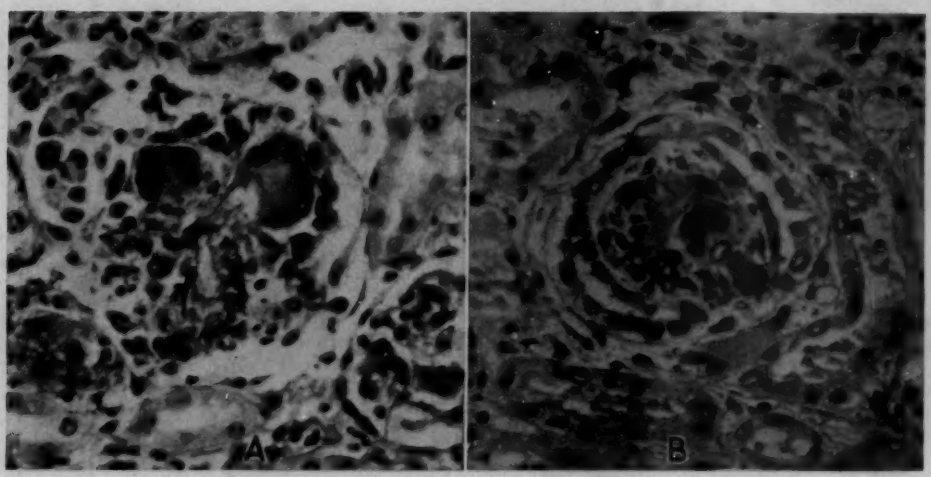

Fig. 3.—*A*, glomerular epithelial giant cell formation in treated monkey 14; *B*, glomerular occlusion and calcification in same monkey; × 450.

infiltration is indicated in the table. Arterial modifications, demonstrable in specimens stained with resorcin-fuchsin and with Mallory's connective tissue stain, were conspicuous by their absence. Mild splitting of the internal elastic membrane and thickening of the intima, to which some authors have drawn attention, were also observed in some of our untreated monkeys and disregarded. Slight thickening of the epithelium of Bowman's capsule was more frequent in the controls, but two layers of cells were very seldom seen. Glomerulonephritis was found in only two treated animals. The injured glomeruli (fig. 3*A* and *B*) were comparatively infrequent, constituting less than 2 per cent of the total number. The similarity in appearance and orientation of the giant cells in figure 3*A* to a giant cell of the "glomerulothels" illustrated by Ran-

derath [16] in his figure 7 is noteworthy. There is a strong resemblance between some of the altered glomeruli and Mallory's [17] figure 427. Calcification (fig. 3*B*) was unusual. No glomerular necrosis of the type and extent illustrated by Billig [18] in her figures 4 and 5 was seen.

Injury seemed to be focused on the renal tubules, particularly on their thick segments (distal convoluted ones). The most consistent change was an increase in nuclear inclusions. We have previously reported [19] their presence in twelve of sixteen treated animals as compared with their occurrence in one of ten normal controls (both renumbered in this paper) and in eighteen of one hundred and seven pathologic controls, monkeys employed in the laboratory for a variety of experiments (which we specified) but not given viosterol. Since doing so, six normal controls have been added of which only two showed inclusions, which gives an incidence of inclusions in three of sixteen treated animals. Most of the inclusions very definitely belonged to Cowdry's [20] type B, which means that they resemble the inclusions in Borna disease in that they are roughly spherical, more or less hyaline bodies, the appearance of which is not accompanied by a margination of basophilic chromatin on the nuclear membrane. See figures 1, 2 and 3 of our previous paper. It was unusual to find in the treated monkeys hypertrophied and desquamated cells possessed of nuclear inclusions with marginated chromatin plus cytoplasmic inclusions as represented in figure 9 of the paper mentioned, though they did occur very sparsely in some of them. The principal sites of the formation of inclusions were the proximal and distal convoluted segments and the beginnings of the collecting segments in the medullary rays. The thin segments were seldom involved. Inclusions were extremely rare in the renal medulla.

Marked nuclear inequality, not observed in the control animals, was often seen in tubules exhibiting the inclusions. The diameters of some nuclei were as much as four times those of the adjacent ones, but the shape and internal structure did not always show a parallel change. The cells possessed of swollen nuclei were often somewhat enlarged, but, examined by ordinary methods, they did not regularly show signs of cytoplasmic injury. Some of the hypertrophied nuclei contained inclusions while others were free from them. The most discrete inclusions sometimes developed in nuclei that were but little enlarged. In a few monkeys, no. 4 particularly, this nuclear inequality progressed to extreme

16. Randerath, E.: Beitr. z. path. Anat. u. z. allg. Path. **85**:85, 1930.
17. Mallory, F. B.: The Principles of Pathologic Histology, Philadelphia, W. B. Saunders Company, 1914.
18. Billig, E.: Beitr. z. path. Anat. u. z. allg. Path. **85**:717, 1930.
19. Cowdry, E. V., and Scott, Gordon H.: Am. J. Path. **11**:659, 1935.
20. Cowdry, E. V.: Arch. Path. **18**:527, 1934.

nuclear polymorphism (Cowdry and Scott,[19] figs. 3 to 6). When this happened, the number of nuclei was diminished and evidence of cytoplasmic injury was unmistakable.

A systematic survey for nuclear inclusions of the other organs of treated and control monkeys was not made. But monkey 143 showed in bile ducts inclusions of type A like those in the same situation previously reported by Covell.[21] Type B nuclear inclusions were encountered in the adrenal glands of nine of twelve, in the livers of three of nineteen and in the lungs of one of twelve treated monkeys examined. In the controls, on the other hand, they were noted only in the adrenal

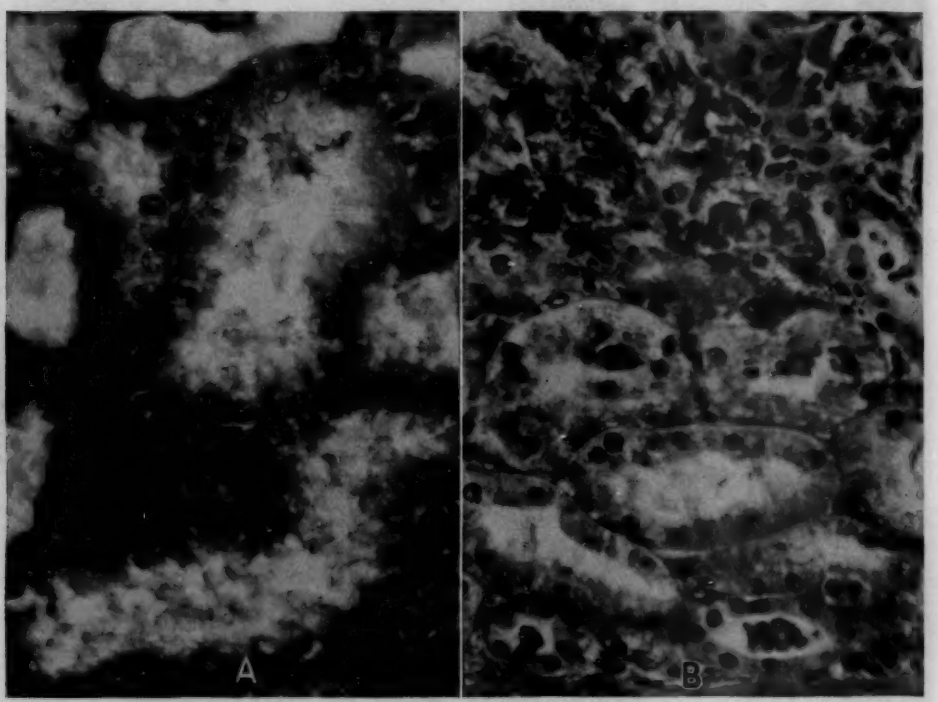

Fig. 4.—*A*, swelling of proximal convoluted segments of the kidney of treated monkey 5. A thick segment (distal convoluted) is represented at the upper margin. Note that its lumen is likewise dilated but that the distal margin of the cells is sharp and clear. *B*, renal tubular necrosis in treated monkey 10. Halfway up the photomicrograph a tubule, cut in two sections, shows the presence of many spherical hyaline granules in the cytoplasm of the dead cells; × 450.

glands of three of sixteen examined. At present we attach no significance to this apparent difference in incidence.

Other signs of injury in the walls of the tubules were not lacking in the treated monkeys, but large areas of necrosis were not observed.

21. Covell, W. P.: Am. J. Path. **8**:151, 1932.

A mild swelling of the proximal convoluted segments is shown in figure 4 *A* (monkey 5), cloudy swelling with cytoplasmic granule formation in figure 4 *B* (monkey 10), necrosis of the first part of a collecting tubule in a medullary ray with heavy luminal invasion of leukocytes in figure 5 *A* (monkey 10) and necrosis without leukocytic invasion in figure 5 *B* (monkey 14). Heavy leukocytic invasion of one distal convoluted segment in monkey 18 was also noted. Giemsa-stained preparations of kidneys of these three monkeys failed to reveal the presence of any bacteria suggestive of an ascending infection. Hyaline casts were fairly frequent in treated monkeys 10 and 19 and rare in control monkeys C and U. With the casts was a good deal of cellular débris. Brightly basophilic material, evidently containing a high percentage of calcium, was conspicuous in the tubular lumens and rather less so in the epithelial cells of monkey 14. These deposits were not confined to the corticomedullary margin, in which Gough and others [22] found calcification in rats given calciferol.

Hypernucleation of tubules with many mitoses in monkey 4 and mitoses without noticeable hypernucleation in monkey 5 indicate a regenerative process. The injury and repair (when present) centered roughly in the segments in which nuclear inclusions were most prone to occur.

Other Modifications.—Special attention was paid to the thyroid glands of the monkeys and numerous measurements were made of the size of the follicles, the height of the epithelial cells and other features. Considerable individual variation was present, but this was approximately equal in the treated and the control series. Toxic changes like those described and illustrated by Kellner [23] for rats were lacking.

Calcification of the adrenal gland was impressive but occurred to about the same degree in the controls. The calcium-rich masses were dense, often laminated and somewhat resistant to sectioning. A few calcified primordial follicles were found in the ovary of one (4) of six treated females. None were seen in the ovaries of four controls, though one of these (N) exhibited traces of calcium in a corpus luteum. The limitation of concretions to the prostate of a control (U) is without significance because the animal was older than any of the treated ones. Only the kidney of treated monkey 14 showed finely divided and rather diffuse deposits of calcium in the tubular lumens and occasionally in the glomeruli, but this monkey had received more of the concentrated preparation of viosterol than any of the others. No traces of calcification of the gastric mucous membrane such as that described by Herzenberg [24] for rats and Laas [25] for rabbits were encountered. Obviously calcification was not a feature of the monkeys treated in the manner specified and on the diet given.

22. Gough, J.; Duguid, J. B., and Davies, D. R.: Brit. J. Exper. Path. **14**: 137, 1933.
23. Kellner, B.: Virchows Arch. f. path. Anat. **288**:491, 1933.
24. Herzenberg, H.: Beitr. z. path. Anat. u. z. allg. Path. **82**:27, 1929.
25. Laas, E.: Virchows Arch. f. path. Anat. **278**:346, 1930.

Epithelial necrosis, desquamation, leukocytic infiltration and hemorrhage were observed about equally in treated monkeys and controls, namely:

Fundus—slight in one (14) of thirteen treated; slight in one (D) of sixteen controls

Duodenum—severe in four (1, 2, 17 and 19) of four treated; severe in one (D) and slight in six (E, G, H, I, N and V) of fourteen controls

Ileum—severe in three (1, 2 and 16) of ten treated; severe in three (C, D and M) of fifteen controls

Colon—absent in all of ten treated; slight in one (D) of sixteen controls

In all of our autopsies the entire gastro-intestinal tract was split open and minutely examined in the gross before tissues were taken. The lesions did not

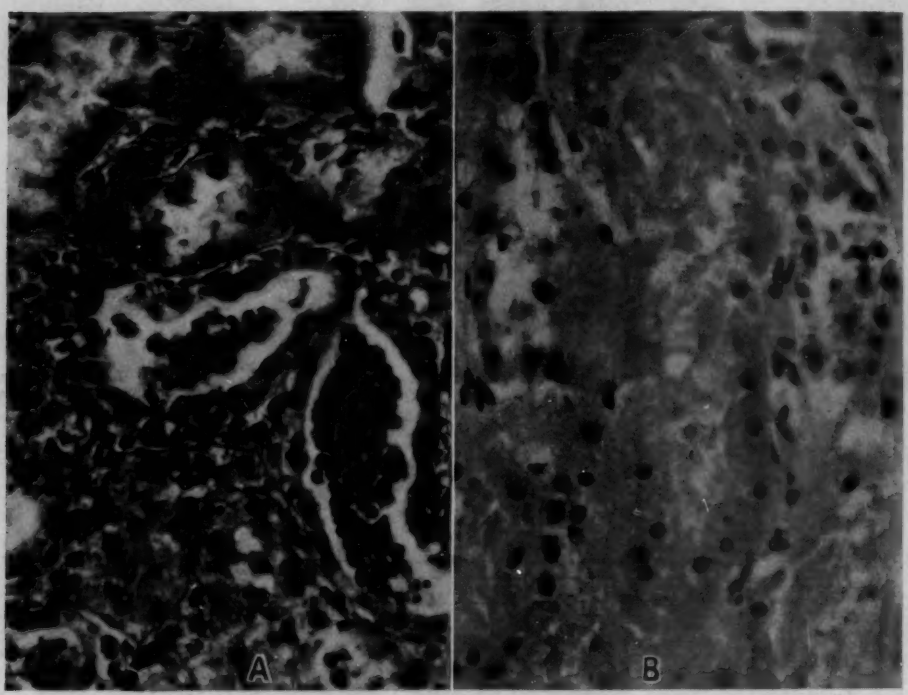

Fig. 5.—*A*, renal tissue from treated monkey 10. In the upper left hand corner, a but slightly injured proximal convoluted segment is shown. In the center and below to the right, two thick segments contain many polymorphonuclear leukocytes, a few lymphocytes, desquamated epithelial cells and much débris. The act of invasion is represented in the lower wall of the centrally placed tubule. *B*, renal tissue showing tubular necrosis without accompanying leukocytic infiltration in treated monkey 14. Long stretches of the vertically placed tubule are without nuclei, and most of the remaining nuclei are contracted and pyknotic; × 450.

appear to be caused by the spirochetes mentioned later in this paper. Had we been content with a smaller number of monkeys we might have taken them in such an order as to lead us to consider our results confirmatory of the observation

of gastro-intestinal ulceration and hemorrhage reported by Taylor and his associates [26] in well conducted experiments on dogs.

Hyalinization of the malpighian corpuscles of the spleen was likewise about the same in the two series. Herxheimer [27] reported somewhat similar changes in the spleens of 15 per cent of untreated children under 10 years of age. The central arterioles of control monkey N were unusually tortuous. Weights of spleens were not taken, so we do not know whether they were atrophied in our monkeys as Kreitmair and Moll [28] (their fig. 1) found them to be in mice. But histologic studies showed no signs of atrophy.

Eosinophilia of the intima of the pulmonary artery was found only in one control. Torhorst [29] and Ljungdahl,[30] who paid very particular attention to the intima of the pulmonary artery, made no reference to such a change. No mast cells [31] were seen in the intima of any of our specimens. Penetration of the parathyroid gland by eosinophils was marked in two treated monkeys. Other tissues of the same monkeys did not exhibit a similar eosinophilia. Our monkeys had comparatively few animal parasites. None were seen in the monkeys showing this eosinophilia (K, 4 and 10), though they may have been present. Trematodes were noted in the buccal epithelium of one (A) of nineteen and helminths in the intestines of F and M. No explanation of the eosinophilia is offered.

In eleven of the nineteen treated monkeys the myocardium showed infiltration with lymphocytes, macrophages and rarely polymorphonuclears. The adjacent cardiac muscle often exhibited acidophilic degeneration. It was when many injured fibers were being absorbed that macrophages were most numerous. Of the controls only two showed focal lymphocytic infiltration. This was of mild degree and was not accompanied by muscular degeneration. We attribute these cardiac changes in the treated animals to the repeated puncture of the heart in the collection of blood for chemical analysis—a procedure not resorted to in the controls.

Supplementary Experiments.—The foregoing observations relate to the concentrated preparation of viosterol. Monkey 12 was given large doses of viosterol of the usual potency to discover whether the corn oil, which is used as a vehicle for vitamin D in both products, could by any chance be harmful. A total of 871 cc. was administered within a period of thirty-five days. Intimal lesions of both types were observed in the abdominal aorta, but they were so mild—not exceeding in degree those found occasionally in untreated monkeys—that their presence did not indicate toxicity on the part of the corn oil.

Only two monkeys of the species Cebus fatuellus were available, but it seemed worth while to make a preliminary test of their response to the concentrated preparation of viosterol. They received six doses of 5 cc. each within two hundred hours. Intimal thickenings and renal

26. Taylor, N. B.; Weld, C. B.; Branion, H. D., and Kay, H. D.: Canad. M. A. J. **25**:20, 1931.
27. Herxheimer, G.: Berl. klin. Wchnschr. **54**:82, 1917.
28. Kreitmair, H., and Moll, T.: München. med. Wchnschr. **75**:637, 1928.
29. Torhorst, H.: Beitr. z. path. Anat. u. z. allg. Path. **36**:210, 1904.
30. Ljungdahl, M.: Kleinen Kreislaufs, Weisbaden, J. F. Bergmann, 1915.
31. Ssolowjew, A.: Virchows Arch. f. path. Anat. **243**:44, 1923.

changes (including inclusions) were apparently lacking in both. The salivary glands, on the other hand, showed nuclear inclusions, intense local infiltration with lymphocytes, macrophages and some polymorphonuclears, accompanied by variable necrosis and the formation of large masses rich in calcium. One exhibited a little hyalinization of the malpighian corpuscles of the spleen.

These monkeys differed from the rhesus ones in several particulars: 1. They were of smaller size. 2. The ossification of the tracheal cartilages and the active spermatogenesis in the testicles indicated that they were more mature than most of the rhesus monkeys. 3. The presence of nuclear inclusions in the salivary glands, which were characterized by scarcity and marked uniformity in size, suggested the preexistence of a quiescent virus apparently not present in this situation in the rhesus monkeys. 4. Spirochetes were absent in the gastric mucous membrane whereas every rhesus monkey examined showed these organisms in abundance, especially in the cytoplasm of the parietal cells and in masses in the lumens of the necks of the glands, often so dense as to act as plugs. 5. They were fed some calcium gluconate in tomato juice, which was never given to the rhesus monkeys.

COMMENT

Our experiments gave practically negative results since the animals did not reveal changes like those which are well known to occur after the administration of heavy doses of viosterol. To find such alterations was, however, not our object. The significant fact is that alterations not hitherto described followed the administration of amounts of a concentrated preparation of viosterol which do not cause hypercalcemia under the conditions specified. Monkeys 4, 10, 14 and 18 must be disregarded in trying to reach an answer to the question raised at the beginning of this paper, because with them we overstepped the mark in dosage with the result that the blood calcium did rise, though only for a few days, and in monkeys 4 and 14 some clinical signs of injury were noted. Otherwise it appears from the balance sheet of table 3 that: (1) the tendency to thickening of the intima of the larger arteries noted in a few untreated monkeys was accentuated; (2) the incidence of some lesions of the kidney was slightly increased, and (3) the parathyroid glands exhibited on the whole a noticeably greater washing out of basophilic cytoplasmic material. Most of the treated monkeys were immature and thus comparable with children. But they were subjected to repeated withdrawals of comparatively large amounts of blood (about 10 cc. at a time), which introduced a complicating factor.

What would have been found had the monkeys been examined not immediately but several years later is difficult to predict. The intimal injuries might have regressed or progressed. The renal lesions might

have been repaired, for regeneration was evident in two animals. It is unsafe to hazard an opinion as to why an increase in nuclear inclusions took place in the cells of the renal tubules. The rather obvious interpretation that it suggests the activation of a virus normally present in a latent state in the kidneys of Macacus rhesus would have greater justification had all the inclusions been of type A. The fact that inclusions of type A were observed in the intrahepatic bile ducts of only one of all the treated monkeys examined means nothing either way. The bile ducts were not injured by the treatment as the renal tubules were, so that there was no histologic evidence of a change that might lead to activation. In the literature we have been unable to find any report on the examination of the kidneys of Macacus rhesus fed viosterol. Numerous papers dealing with other species make no allusion to nuclear inclusions. Evidence of the existence of a virus in a latent state is lacking except for rats [32] and human infants,[33] which are on a par with Macacus rhesus [34] in that nuclear inclusions suggestive of virus action are known to occur in a small percentage of each group. The kidneys of rats treated with viosterol have been repeatedly examined by competent investigators, but the discovery of an increase in the incidence of nuclear inclusions has not been announced. Renal lesions in children given large doses of viosterol have also been studied without any mention being made [35] of nuclear inclusions. That an increase in inclusions was present and overlooked is hardly likely, for well formed nuclear inclusions are very conspicuous objects, the significance of which is much debated in current literature.

The decrease in the basophilic cytoplasmic bodies of the parathyroid glands is equally definite so far as it goes. The comparison with the controls loses force because the parathyroid glands of only seven of the treated animals were examined. The conditions in the remainder are not known. The available evidence is compatible with the conclusion that the material responsible for the local basophilia disappears to a greater or lesser extent as a result of the treatment. It may or may not constitute an antecedent of secretion. The cytology of the parathyroid glands is nicely presented by Marine,[36] who draws attention to a description by Bobeau [37] of certain cytoplasmic granules which he

32. Hindle, E., and Stevenson, A. C.: Tr. Roy. Soc. Trop. Med. & Hyg. **23**:327, 1929.

33. Farber, S., and Wolbach, S. B.: Am. J. Path. **8**:123, 1932.

34. Cowdry and Scott.[19] Farber and Wolbach.[33]

35. Bamberger and Spranger: Deutsche med. Wchnschr. **54**:1116, 1928. Putschar, W.: Ztschr. f. Kinderh. **48**:269, 1929. Tu-Tunji, D. F.: Lancet **1**:53, 1931. Klausner-Cronheim, I.: Deutsche med. Wchnschr. **56**:1566, 1930. Thatcher, L.: Edinburgh M. J. **38**:457, 1931.

36. Marine, David: The Thyroid, Parathyroids and Thymus, in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, Inc., 1932, vol. 2, p. 798.

37. Bobeau, G.: J. d'anat. et physiol. **47**:371, 1911.

regarded as secretion granules. We have been unable to confirm Bobeau's work. As yet we have not made any Golgi or mitochondrial preparations, nor have we stained for glycogen. The status of the basophilic bodies is to some extent comparable to that of the renal intranuclear inclusions. They have been seen in the parathyroid glands by other workers, but a change in them has not been described despite numerous studies of these glands in animals given much viosterol. Instead, alterations which are perhaps more radical and less delicate have been reported. The literature on the parathyroid glands has been summarized by Castleman and Mallory,[38] who on the basis also of much original work stated that (in man) "The clear cell hyperplasias prove that a physiological stimulus can convert every parathyroid cell into the wasserhelle type." Sekiguchi [39] observed an increase in clear cells in rachitic rats. We noticed in our treated monkeys a slight increase in the water-clear type as well as in the oxyphils. Two of the clear cells are shown in figure *2 A*, which illustrates a parathyroid gland from which the cytoplasmic basophilic bodies have disappeared. It seems not unlikely that depletion of basophilic bodies is the first stage in the formation of water-clear cells and that these same basophilic bodies are the long sought antecedents of parathyroid secretion. Until cytologists pay the close attention to the parathyroid glands which their functional importance demands, this will remain only a challenging possibility.

We do not wish to emphasize the other less frequently met with lesions which we have described in monkeys given small amounts of the concentrated preparation of viosterol. But we would point out that the actual changes may have been more extensive than the observed ones. The intimas of only the larger vessels were examined in the gross. Histologic preparations were limited to a particular part of each artery. Had they been more widely spread, alterations might have been discovered in animals reported as not showing changes, and more severe lesions, which we did not happen to encounter, might have been detected in the others. Similarly, the study of more blocks of tissue from each organ might well have revealed other significant modifications.

When we arranged our animals in a graded series depending on the total amounts of the concentrated preparation of viosterol administered, we could not establish any consistent correlation between heavy dosage and maximum degree of nuclear inclusion formation, of parathyroid change or of any of the other histologic modifications. It will be recalled that Reed and his associates [40] found that in dogs the increase in the

38. Castleman, B., and Mallory, T. B.: Am. J. Path. **11**:1, 1935.

39. Sekiguchi, S.: Jap. J. Exper. Med. **8**:421, 1930.

40. Reed, C. I.; Dillman, L. M.; Thacker, E. A., and Klein, R. I.: J. Nutrition **6**:371, 1933.

calcium of tissue, as determined by direct chemical analysis, was not correlated with increase in the viosterol dosage but seemed to be conditioned by some unknown individual factor or factors. Others have had a similar experience.

To relate our maximum and minimum doses to the amounts given children is a difficult matter. On the discovery of the therapeutic value of viosterol, large amounts were administered; but fear of the consequences grew, and the pendulum swung perhaps too far in the opposite direction. Now a tendency to give slightly more is apparent. We have not been able to find any authoritative statement of what the therapeutic dose for infants should be when the concentrated preparation of viosterol considered in this paper is used. We cannot calculate the number of U. S. P. X1 units of vitamin D which it should supply from the number contained in amounts of cod liver oil considered effective, because the cod liver oil contains other substances in addition to vitamin D, notably vitamin A, so that it is qualitatively as well as quantitatively different from viosterol. But the dosage for viosterol of the usual potency is given in "New and Nonofficial Remedies." It varies from 8 to 20 drops per day. Reed [41] wrote that 1 drop of viosterol of a potency of 920,000 U. S. P. X1 units of vitamin D per cubic centimeter contains 30,000 units of vitamin D, and 1 drop of viosterol of the usual potency, only 300. Consequently, if the concentrated preparation were substituted for viosterol of the usual potency, one one-hundredth of the approved dose would be indicated. Calculated on the basis of 30 drops per cubic centimeter, the dose for infants would be between 0.0026 and 0.006 cc. Harris [42] placed the maximum curative dose at 5,000 units of vitamin D per day for children from 1 to 2 years of age. Calculated on the basis of 1 cc. of viosterol containing 920,000 units of vitamin D (the figure given by Reed), the maximum curative dose would be 0.0054 cc. This is practically the same as 0.006 cc. In weight our monkeys averaged 2,783.2 Gm., and the infants might easily be twice or four times as heavy. Assuming that young monkeys react as children are supposed to do, we have given doses of the concentrated preparation of viosterol far in excess of what might be expected to be therapeutic, but the level of blood calcium was not raised except in the four animals specified. This rather points to the conclusion that the response of monkeys is quantitatively different from that of children and that they show perhaps less susceptibility to viosterol as measured by blood changes. The difference, however, may be more apparent than real. The object of the specified dosage for infants is to raise the blood calcium to normal when it is abnormally low, not to produce hyper-

41. Reed, C. I.: J. A. M. A. **102**:1745, 1934.
42. Harris, L. J.: Brit. M. J. **2**:367, 1933.

calcemia. Apparently no figures except ours are available for the normal blood calcium of young rhesus monkeys. If these figures are substantially correct, the blood calcium of our monkeys was not low to start with. If it had been, a dosage more nearly comparable to that recommended for infants might have raised it.

We pass now to older children and adults. Reed [40] has summarized his experience with three hundred persons ranging in age from 7 to 72 years. He gave as much as 3 cc. of viosterol (containing 920,000 units of vitamin D per cubic centimeter) daily for a period of five days "without the slightest evidence of injury." The weights of the particular patients were not cited. If they were adults weighing on the average 150 pounds (68 Kg.), the dose would have been 0.0434 cc. per kilogram; if children, very much more per kilogram, and our minimum dose in monkeys would more nearly approximate the latter.

Only two monkeys of the species Cebus fatuellus were treated,[43] and they were by contrast adults; but the striking differences from monkeys of the species Macacus rhesus which they exhibited constitute a warning against the conclusion that because the latter reacted as described children are likely to follow suit. Other fundamental differences in the susceptibility of the species to irradiated products are considered fully and critically by Laurens,[44] Anitschkow [45] and others. Our experiments, therefore, do not permit even an indirect answer to the question of what the effect of small doses of a concentrated preparation of viosterol may be on children. They do indicate nevertheless that in monkeys slight thickenings of the intima and severe renal lesions occur in the absence of clinically recognizable signs of injury, which means that the possibility should be entertained that children, also, may suffer hidden injuries not manifested clinically.

SUMMARY

Before treatment of young monkeys (Macacus rhesus) on the diet specified, the average amount of serum calcium in males was 11.62 Gm. and that in females 10.99 Gm., while the average amount of serum phosphorus in males was 5.96 Gm. and that in females 6.02 Gm. per hundred cubic centimeters of serum.

During the administration of a concentrated preparation of viosterol in the amounts described, the average amount of serum calcium remained about the same, but the average amount of serum phosphorus increased to 7.75 Gm. in males and to 7.44 Gm. in females.

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43. Cowdry, E. V., and Scott, Gordon H.: Am. J. Path. **11**:647, 1935.

44. Laurens, Henry: The Physiological Effects of Radiant Energy, New York, The Chemical Catalog Company, Inc., 1933.

45. Anitschkow, N.: Experimental Arteriosclerosis in Animals, in Cowdry, E. V.: Arteriosclerosis, New York, The Macmillan Company, 1933, p. 271.

Histologic modifications described in the literature as resulting from excessive dosage with viosterol were inconspicuous. The lesions found in Macacus rhesus were divisible into two classes: The first comprised those some representation of which was detected in the control untreated monkeys but which occurred more frequently or in an intensified form in the treated animals, namely, intimal thickenings, nuclear inclusions in the cells of the renal tubules and reduction of the cytoplasmic basophilic bodies of the parathyroid glands. The second comprised alterations not seen in any of the controls, namely, calcification and fragmentation of the internal elastic membrane of the left radial artery of one monkey (14), infiltration of parathyroid glands with eosinophils in two (4 and 19), renal tubular invasion by polymorphonuclear leukocytes in three (4, 10 and 18), renal tubular degeneration in two (4 and 5) and calcification in one (14).

Except in the case of monkeys 4 and 14 there was no clinical reason to suppose that the treatment had been injurious; indeed, the treated animals appeared to be in excellent condition; monkeys 4, 10, 14 and 18, however, exhibited a terminal rise in serum calcium over 17 mg.

Two mature monkeys of the species Cebus fatuellus reacted so differently from those of the species Macacus rhesus as to indicate the unjustifiableness of making a prediction as to what may be happening in children who are given viosterol and seem to be uninfluenced or improved by it.

MORPHOLOGY OF PROSTATIC CORPORA AMYLACEA AND CALCULI

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The corpora amylacea found in the glandular acini of the prostate have been known since the time of the earliest gross and microscopic studies of this organ and have been the subject of some investigation, but a single correlated morphologic and chemical study has not been attempted. In addition to the direct information on the pathologic changes in the prostate which such a study as this should supply, there are many probabilities as to the general formation of concrements [1] which may be inferred or deducted from an investigation of the prostatic corpora amylacea. For convenience, the material has been divided into two communications, one on the morphologic phases and one on the chemical phases. The isolated and incomplete character of the literature renders it advisable to consider the previous investigations throughout the body of the paper rather than to devote a separate section to this topic.

GENERAL FEATURES

Even cursory examination has shown every anatomist and pathologist that concrements are found in the prostate in increasing number with increasing age. They vary in size from microscopic bodies to large calcified masses from 2 to 4 mm. in diameter, and rarely a single calculus may entirely replace the prostatic parenchyma as in cases reported by Podlasky and Elcorin [2] and MacKenzie and Seng.[3] The small, soft

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This investigation was initiated at the suggestion of Prof. J. Erdheim while I was a National Research Fellow in Medicine in Vienna. The material consists of 678 unselected prostates collected under Professor Erdheim's direction. The preparation of the large number of sections was possible through a grant from the Committee on Grants-in-Aid of the National Research Council. The materials and equipment were supplied by Western Reserve University.

1. The group of words used somewhat indiscriminately in reference to concrements is used in this paper as shown in the following sentence: "The concretion of protein and other substances produces a concrement which may be infiltrated with inorganic salts to form a calculus."

2. Podlasky, H. B., and Elcorin, D. V.: Urol. & Cutan. Rev. **31**:42, 1927.

3. MacKenzie, D. W., and Seng, M. I.: J. Urol. **12**:243, 1924.

concrements are designated corpora amylacea, while there has been an attempt especially in the urologic surgical literature to separate the large, calcified, clinically manifested calculi.[4] As will be shown in a later section, it is practically certain that the two types are directly related and have the same genesis. Grossly the corpora vary from pale yellow to dark brown or black, and microscopically (unstained section), from colorless to dark brown. The calculi are usually brown but may show white or gray areas. In ground sections these are composed largely of crystalline inorganic salts with little organic matrix. Typical corpora may be found in any portion of the prostate, but they are most abundant in the cephalad portion of the posterior lobe and in the larger ducts and adjacent acini of the lateral lobes. They are infrequent in the middle lobe and rare in the anterior lobe. As is well known, they are not as abundant in the acini of a prostate showing benign enlargement as in normal acini. The larger calculi are usually found in large cystic spaces formed by dilatation or coalescence of the ducts, but rarely they are located in the glandular parenchyma. Observation of entire corpora and reconstruction from serial sections show a general round or elliptic shape, but markedly irregular and branched forms may be found. Many of the smaller corpora, but in this study rarely the calculi, show faceted surfaces. The consistency varies from soft plastic to firm. The former represents little more than agglutinated, partially inspissated organic material, while the latter forms fracture lines when crushed and frequently separates along the concentric layers. These two extremes may be combined in the same corpus; such a corpus has a hard external layer and a soft plastic or perhaps even fluid center, or the reverse. The surface of the single round or elliptic corpus is smooth, and the irregular corpora show no nodularity except as determined by the shape of the acinus. Calculi, on the contrary, present typically a nodular surface. The nodules vary from 200 microns to 1 mm. in diameter and are elevated above the surface about one-half of their diameters (fig. 1 *A*).

THE MORPHOLOGY OF THE TYPICAL CORPUS[5]

As described, the typical corpus is a smooth spheric light yellowish-brown body about 250 microns in diameter. In microscopic section it is a round colorless disk which stains lightly acidophilic with the eosin series of dyes, dark blue with nile blue sulfate, very faintly orange with sudan III or scarlet red and red or green with the acid fuchsin-light

4. Throughout the urologic literature prostatic calculi are divided into exogenous and endogenous. The former are urinary calculi which by retrograde movement come to lie in the prostatic ducts. These are not discussed in this paper.

5. Although paraffin sections were prepared, the greatest reliance was placed on gelatin-embedded frozen sections. As a routine stain, hematoxylin (Ehrlich's) and sudan III were employed.

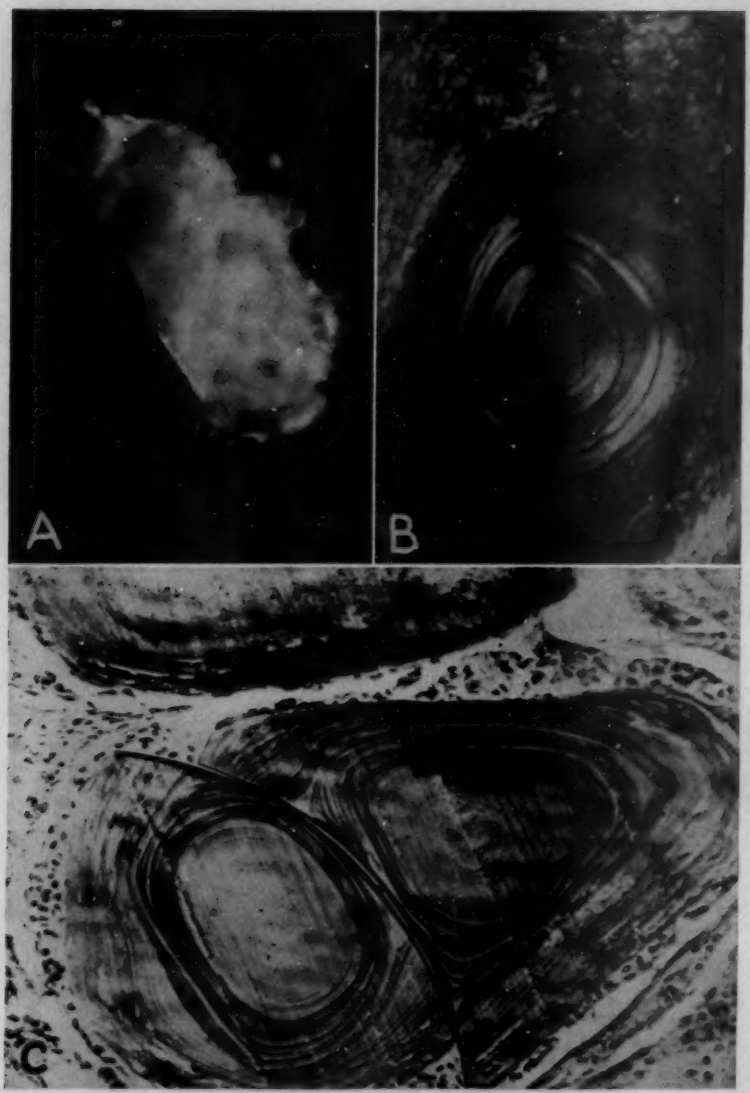

Fig. 1.—*A*, a photograph of a small prostatic calculus, made by reflected light. Note the small nodules over the surface. These have the same consistency as the calculus. *B*, a typical corpus photographed with polarized light and crossed Nicol prisms; × 170. Note that some layers are doubly refractile and that others are not. The extinguished cross corresponds to the planes of the polarizer and analyzer. The illuminated areas about the corpus represent collagen fibers which are weakly anisotropic. *C*, faceted surfaces on adjacent corpora; × 410. Note that the peripheral portions are eroded since the concentric lines do not turn onto the faceted surfaces. On the lower border of the right hand corpus there is an additional flattened surface with closer approximation of the lines.

green trichome stain of Masson.[5a] With iodine and iodine and sulfuric acid the reaction is typically brown and bluish green, but this is extremely variable and some parts of the corpus may remain unstained or may not change color with sulfuric acid. The iodine stain furnishes some evidence of changes in composition with increased age. The more peripheral, less dense portions usually stain some shade of green with iodine while the more central, denser areas stain brown. Where there is abundant pigment, it is difficult to determine how much of the brown color is pigment and how much is stain, but it is probable that the pigmented areas react little if at all with iodine. This difference in reaction between material from different layers may be the expression of a different physical state, but with other evidence of secondary changes to be presented later it is interpreted as a change in chemical constitution. With the metachromatic aniline dyes (gentian violet, methyl violet), they usually show the metachromatic color, but again whole corpora or parts of a corpus may fail to stain.

The internal structure in its simplest form is homogeneous or finely granular with or without a central nidus. However, in the majority of corpora there are one or more concentric lines which divide the corpus into layers. These lines stain more intensely than the intervening substance and so far as can be determined by morphologic methods represent a condensation of material and not a difference in chemical composition. Schade [6] explained this concentric lamination on the basis of Liesegang's rings. Such a hypothesis requires the assumption that after the first material within the acinus forms into a gel-like mass there appears a new substance which reacts with and precipitates a substance in the original mass. There is no evidence for or against this assumption, but in harmony with the generally accepted theory that corpora are formed from desquamated epithelial cells and secretion it seems unlikely that conditions are correct for the formation of Liesegang's rings. On the contrary, the facts are more consistent with the identification of the concentric lines with progressive phases of growth. The direction and separation of the lines on faceted surfaces clearly indicate a decrease in the velocity of growth (fig. 1 *C*). As further evidence, there is an apparent difference in the chemical composition of different layers, shown by a difference in the staining reaction and double refraction.

The typical corpus when examined whole or in section with polarized light and crossed Nicol prisms shows the polarization cross with the lines of extinction parallel to the planes of the analyzer and polarizer

5a. Masson, P.: J. Tech. Methods **12**:75, 1929. Foot, N. C.: Stain Technology **8**:101, 1933.

6. Schade, H., in Alexander, J.: Colloid Chemistry, New York, Chemical Catalog Company, 1928, vol. 2, p. 803.

(fig. 1 *B*). The usual organic solvents, alcohol (50 per cent, 70 per cent and absolute), ether, acetone, chloroform, pyridine, benzene and zylene, and the weak organic and dilute (5 per cent) inorganic acids have no effect on this phenomenon. However, dilute alkalis, especially ammonia, effect changes which result in the disappearance of double refraction. This is true for both the entire unfixed corpus and for fixed sections, and thus it may be concluded that the double refraction is not due to a lipid.[7] The chemical evidence that nucleic acid and adenine or guanine or both are present in the corpus, to be presented in the succeeding paper, led to the conclusion that crystalline ammonia-soluble adenine is the substance responsible for double refraction. Further evidence for this view is furnished by the histochemical observation of precipitation of silver along the radial lines after proper treatment.[8] Not only does the precipitate occur along definite architectural lines, but the corpus thus prepared no longer shows double refraction, indicating that the anisotropic compound has lost its identity by interreaction with silver salts. On this evidence it is proper to conclude that when the matrix becomes of a certain density and age crystals of adenine appear as definite morphologic structures and the corpus shows radial striae (fig. 2 *A*).

SECONDARY CHANGES

In every prostate with any number of corpora, a considerable number will show a reticulated or vacuolated center of lesser density (fig. 2 *B*). It seems impossible to assume that this represents the deposition of a firm compact layer around an original plastic or liquid center. The assumed histochemical evidence of the presence of purines and the definite chemical evidence of nucleic acid in the corpora render it much more likely that this center represents a secondary change due to the hydrolysis of nucleic acid. In the chemical portion of this report, data will be presented to show that the nuclease enzymes necessary for this hydrolysis are present in saline extracts of the prostate. This conception involves first the hydrolysis of some or all of the nucleic acid or of the nucleotides with the formation of the purines in the form of small crystals. As has been shown for gallstones, there is a rearrangement of the crystal structure with growth of a few crystals at the expense of the others. This results in the visible radial striae. These larger crystals may undergo further hydrolysis, or the original hydrolytic

7. The word "lipid" is used throughout this paper in preference to "lipoid" and "fat" in keeping with the report of the International Committee on Biochemical Nomenclature.

8. On the basis that the purines (adenine, guanine, etc.) form insoluble silver salts, frozen sections were washed in distilled water, placed in 5 per cent ammoniacal silver carbonate for two minutes, washed, placed in hydrogen sulfide for five minutes, washed and mounted in gelatin.

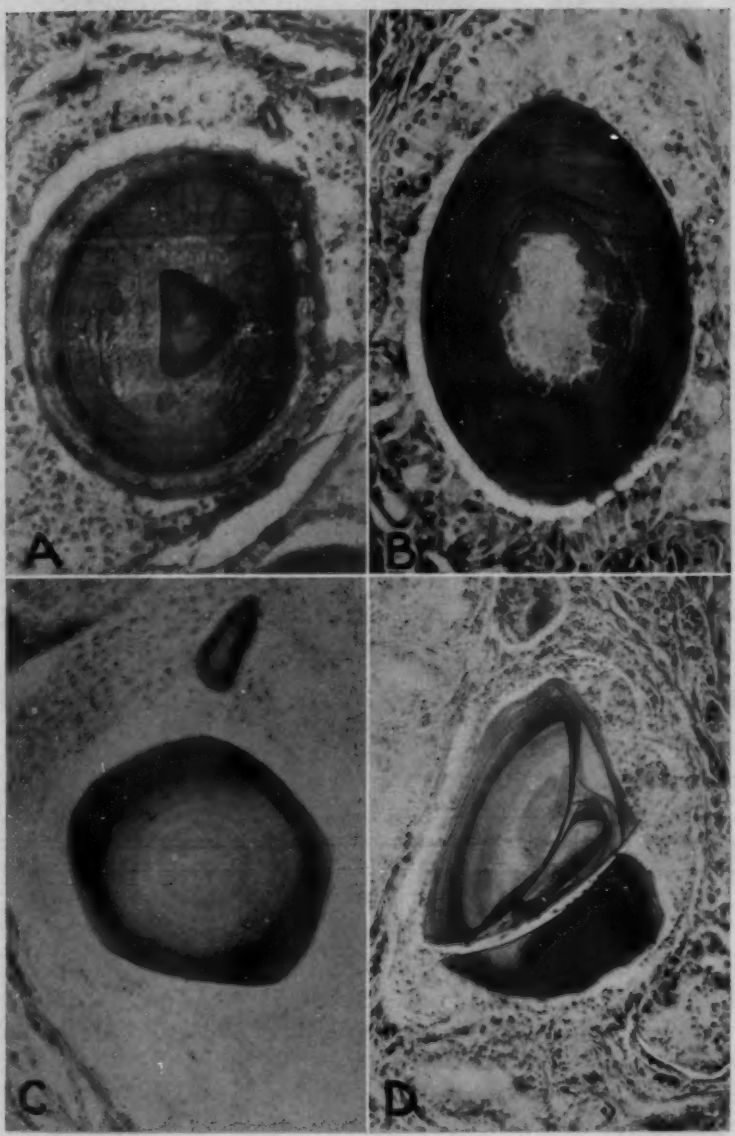

Fig. 2.—*A*, a corpus with radial striae in the center and concentric lines in the periphery; × 150. *B*, a corpus with a central amorphous mass, probably the result of secondary softening; × 270. *C*, a corpus with concentric layers about the fractured parts of an older corpus; × 200. Note also the facets and irregular concentric lines. *D*, folding of the middle layer of a corpus, probably the result of a compression of the plastic mass; × 340.

process may proceed by a direct route to completion and the formation of a fluid vesiculated center such as that shown in figure 2 *B*.

Aschoff and Bacmeister [9] showed that this central crystal and liquid structure may weaken a gallstone so that spontaneous fracture may occur. Confirmation of this observation is seen in a corpus with the structure shown in figure 2 *C*. In the central portion there is embedded a portion of the outer layer of an old corpus. Such a figure as this could be produced only by the process of central softening and fracture of the peripheral portion. Further evidence that the central fragment is real is furnished by the independence of the crystal line structure of the central and peripheral portions when examined with polarized light and crossed Nicol prisms. It is extremely difficult, if not impossible, to submit morphologic evidence of spontaneous fracture before separation and recovering of the fragments. In a recent paper Sprafke [10] subscribed to this assumption of central softening but offered no hypothesis for it other than that it represents a colloidal *Entquellung*. He illustrates a common observation, namely, that of epithelial cells and rarely connective tissue growing in through a single fracture and filling the central cavity. The physical force for the production of a fracture is available in the abundant smooth muscle of the stroma.

Another related phenomenon is the deformation of a corpus and subsequent formation of new peripheral layers. In figure 2 *D* is an elliptic corpus deformed and bent at two points. The points of strain are indicated by folds and wrinkles in the middle layer. Such a picture as this could represent an artefact if it were not for the presence of a more peripheral, closely apposed nonwrinkled layer.

Although morphologic evidence of secondary softening is of necessity limited to partial destruction, there is no theoretical consideration to prevent the extension of the process to entire corpora and to their complete dissolution. It is possible that some of the acidophilic granular débris seen in acini is the result of this process.

Corpora in the prostate from a person in the eighth or ninth decade of life are not infrequently found isolated in the stroma and not surrounded by epithelium. The morphologic evidence indicates that this result may be obtained by either one of two processes. The epithelial atrophy may be of the senile type, or it may be the result of pressure from the corpus. Not infrequently in the latter case and rarely in the former, multinucleated cells are formed about the bare corpus. Their origin is in doubt, but on occasion they are observed in direct relation to cells that are clearly epithelial, and hence they can be accepted as derivatives of the former acinar epithelium. They have little effect on

9. Aschoff, L., and Bacmeister, A.: Die Cholelithiasis, Jena, Gustav Fischer, 1909. Aschoff, L.: The Origin of Gall Stones, in Lectures on Pathology, New York, Paul B. Hoeber, Inc., 1924, p. 206.

10. Sprafke, H.: Frankfurt. Ztschr. f. Path. **45**:191, 1933.

the corpus since no evidence of digestion or of phagocytosis can be demonstrated except a rare smooth shallow excavation under a cell. Wilke [11] came to similar conclusions. With the exception of the characteristic stromal changes of senile atrophy there is only rarely any reaction about the isolated corpora. When reaction does occur, it consists of a very slight lymphocytic infiltration. This indicates that the chemical substances present are inert in the stimulation of fibroblasts and attraction of wandering cells.

Many corpora show one or more faceted surfaces (fig. 1 *C*). It seems improbable that the position of the acinar epithelium would remain fixed a sufficient length of time to allow a faceted surface to form. The only alternative is that two corpora are in contact and by deficiency of growth the facet is formed. The deficiency of growth may be equal on the two corpora, and the apposition surface is then flat. However, growth may occur to a greater extent at the periphery on one corpus and at the center on the other so that interlocked corpora are formed. The apposition may occur between two corpora in the same acinus or in adjacent acini with partial or complete atrophy of the stroma and epithelium between the two. At any time two faceted corpora within a single acinus may fuse by the formation of a peripheral layer about both bodies and between them (fig. 3 *A*). With polarized light and crossed Nicol prisms the crystal structure of each is seen formed about its own center while the crystal structure of the common peripheral layer centers about the entire mass. In serial sections it is easily demonstrated that two large corpora which entirely occupy an acinus will show two or more faceted surfaces. Such a structure as this is possible only if rotation occurs and new appositions are formed from time to time. Aschoff and Bacmeister [9] showed the same process in gallstones. Also in serial sections a single faceted corpus in a large acinus or duct is not infrequently found. This leads to the conclusion that movement along the duct occurs. Naturally the possibility of movement due to the technical preparation cannot be excluded in autopsy material.

Movement and rotation of corpora are further demonstrated in the rare phenomenon of erosion. In a few acini the structure shown in figure 1 *C* is seen, a flat-faceted surface around which the concentric lines do not bend. Such a corpus as this was observed in a serial section and was demonstrated not to be an artefact. With artefact excluded the only explanation is erosion. Two flat-faceted surfaces with the concentric lines typical of a deficiency of growth are worn until the concentric lines end abruptly, and the narrow accretion growth is eroded. Erosion and fracture demonstrate that the consistency of some corpora is more than that of inspissated albuminous material.

11. Wilke: Virchows Arch. f. path. Anat. **211**:165, 1913.

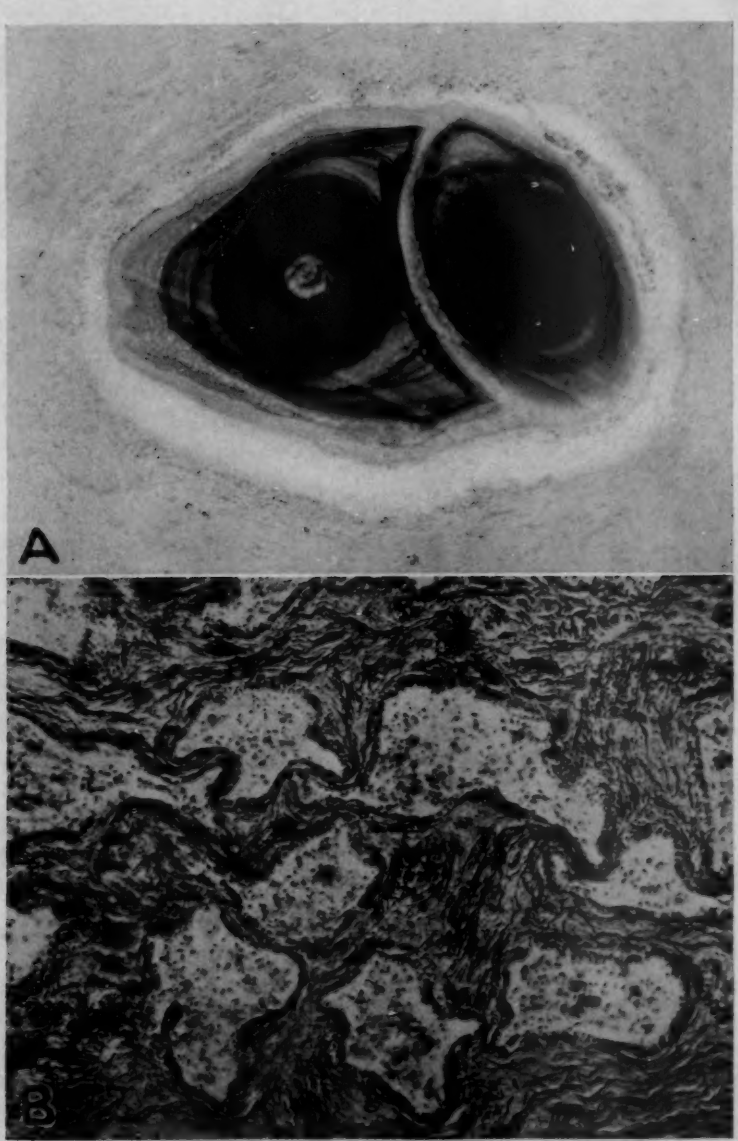

Fig. 3.—*A*, fusion of two formerly separate faceted corpora; × 225. *B*, desquamated cells in the lumen of an acinus with an intact epithelial lining; × 142.

Another secondary change of some importance is the appearance of a brown pigment, especially in the central portions. All the histochemical stains for iron are consistently negative. The pigment rarely occurs in granules or globules and does not stain with silver as melanins do. When whole corpora are extracted with alkaline solutions, as 1.5 per cent sodium hydroxide or 5 per cent ammonia water, the pigment is in large part dissolved. It is adsorbed on the nucleic acid precipitated by alcohol from an alkaline extract (1.5 per cent sodium hydroxide in 10 per cent sodium acetate) and is adsorbed on animal charcoal from strong ammoniacal solutions. Identification of this pigment is not possible at present. It is of interest that corpora with deep brown pigmentation are most abundant in the ducts and about the urethra (probably older) and that secondary central softening is rare in pigmented corpora.

Corpora within an acinus in the lumen of which a purulent exudate subsequently develops are not rapidly digested by the fluid of the exudate, but corpora involved in an area of caseous necrosis undergo rapid dissolution. Simmonds [12] made similar observations in a study of tuberculosis of the prostate.

ORIGIN AND GROWTH

With the preceding knowledge of the structure of the developed corpus and of the secondary changes which may occur in it, it is now possible to understand the genesis of the structure.

Logically it may be assumed that the corpus must be formed from material present in the acinar lumen, and it follows that a careful study of the content of the lumen should disclose the available building materials. It is at once apparent that this material may be divided into two classes: (1) elements clearly a portion of cells or entire cells and (2) elements which give no morphologic evidence of this origin. The former represent desquamated epithelial cells or wandering cells and the lattter secretion and body fluids.

In any prostate from a man over 50 years of age, a variable quantity of intact and partially degenerated cells is found in the acini (fig. 3 *B*). Since the earliest investigations of Thompson [13] these cells have been thought to be an important constituent in the formation of corpora. Björling,[14] H. L. Posner,[15] C. Posner [16] and C. Posner and Rapoport [17] investigated the nature of these cells and concluded that the majority are

12. Simmonds, M.: Virchows Arch. f. path. Anat. **216**:45, 1914.
13. Thompson, H.: Lancet **2**:85, 1857; Tr. Path. Soc., London **12**:139, 1861.
14. Björling, E.: Arch. f. Dermat. u. Syph. **103**:3, 1910; Ztschr. f. Urol. **6**: 30, 1912.
15. Posner, H. L.: Ztschr. f. Urol. **5**:722, 1911.
16. Posner, C.: Ztschr. f. Urol. **5**:161, 1911; Berl. klin. Wchnschr. **46**:254, 1909.
17. Posner, C., and Rapoport, L.: Deutsche med. Wchnschr. **31**:492, 1905.

desquamated epithelial cells. A special stain for oxidase granules shows only a very few cells with these granules, certainly an insufficient number to play any rôle in the genesis of corpora. This does not, however, eliminate the large mononuclear cell and lymphocyte from consideration. On morphologic grounds the cells are similar to the epithelial cells which line the acini and not rarely contain within their cytoplasm secretion granules similar to those in the intact epithelium (fig. 4 *A*). Further, these cells never show definite evidence of phagocytic activity, and migration through the acinar epithelium is rarely observed. It is concluded therefore that the cells found in the acinar lumens are desquamated epithelial cells with a rare wandering cell of the myeloid series. The logical question arises whether or not these desquamated cells represent intravital desquamation. The only answer is that in improperly prepared material postmortem desquamation does occur, but that in carefully controlled material numerous acini with fully intact epithelium are filled with cells. Several acini in a series of thirty sections (from about 10 to 15 microns in thickness) have been followed to demonstrate that this is a constant finding in adjacent portions of a single acinus. Sprafke [10] discussed this point and concluded that desquamation is an intravital physiologic process in the prostate. The occurrence of lipid droplets in these cells is not evidence of intravital desquamation and degeneration since similar droplets may be found in intact cells, but the varied types and stages of degeneration indicate an intravital process. When degeneration is advanced it is not uncommon to find bare nuclei or swollen cells with nuclei which show pyknosis and karyolysis. Karyorrhexis is a rare process under these conditions. Admixed with these cells there is the second type of material mentioned, that not demonstrable as derived from desquamated cells. With the degeneration of the cells a considerable quantity of the homogeneous and granular acidophilic débris is formed, but other acidophilic and basophilic, sudanophilic and nonsudanophilic, highly refractile, large and small granules are found. By comparison with similar granules in intact epithelium it is apparent that this is the morphologic evidence of prostatic secretion. Since these granules are undoubtedly suspended in an albuminous fluid, a portion of the granular acidophilic débris has its origin from secretion. Finally, free and within cells, droplets of lipid are seen. Some of this is doubly refractile. The nature of the prostatic lipid will be considered at greater length in the chemical portion of this report. In summary, the morphologic evidence shows that the prostatic acini are filled with an albuminous fluid, in which there are suspended desquamated epithelial cells in all stages of degeneration, secretion granules, droplets of anisotropic and isotropic lipid and the end-products of cellular degeneration.

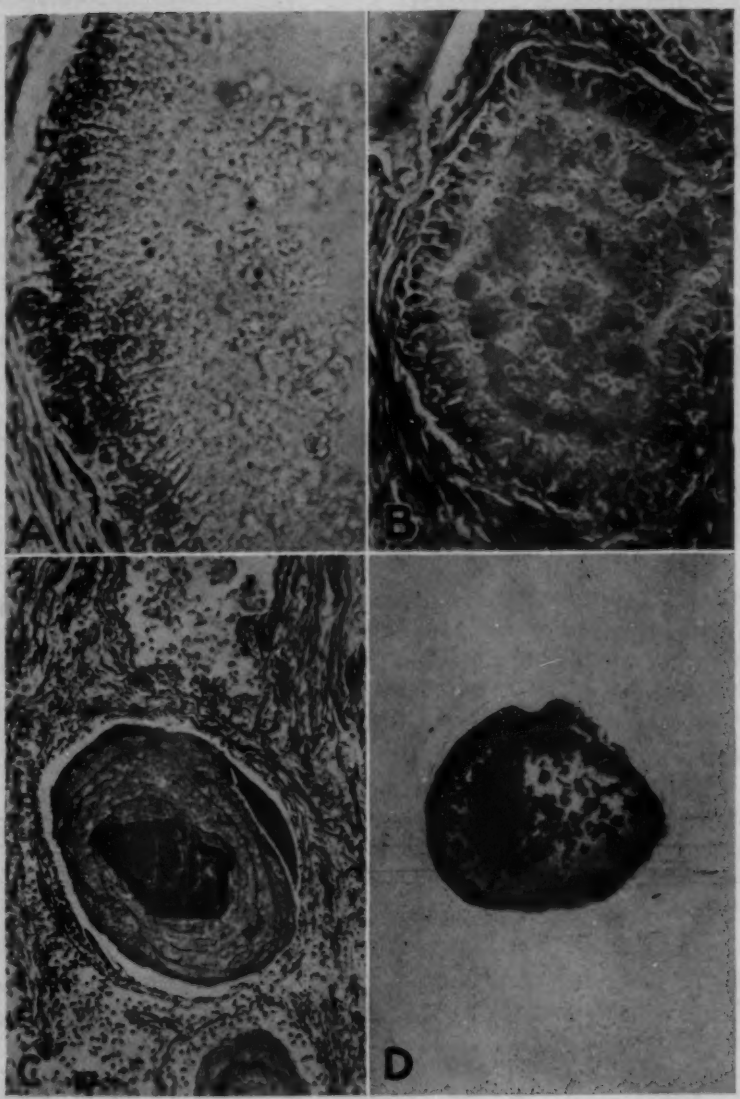

Fig. 4.—*A*, desquamated epithelial cells with secretion granules still intact; × 900. *B*, transition states between swollen degenerated epithelial cells and formed corpora; × 512. *C*, peripheral layers of a large accretion corpus, showing the transformation of cells into the matrix of the corpus; × 220. *D*, early calcification in a corpus; × 140; von Kossa stain. Note the similarity of these structures to the calcospherites of developing bone.

The first step from this material to the simplest single minute corpus is not difficult to demonstrate (fig. 4 *B*). All stages from swollen degenerated but still nucleated cells through the unformed acidophilic granular nuclear-free mass of delimited cellular débris to the compact dense homogeneous corpus are seen. This observation was first emphasized by Eastman [18] and was confirmed by Sprafke.[10] Under these conditions a central nidus is not necessary and is not found, since a single cell is converted into a single small corpus, and careful examination of the entire spheric mass fails to show any central point which differs in structure. In some minute corpora very fine droplets of lipid are indiscriminately distributed, and by comparison with similar droplets found in degenerated cells their origin is easily explained. The reason for this change of the cells into gel-like corpora is not apparent, but it is tentatively accepted that the nucleic acid released by the cellular degeneration is the substance which gels.

The further evolution of these minute corpora is definite in terms of morphology but indefinite in terms of mechanism. Each may remain as a single isolated spheric mass, a group of them may be trapped in a plastic mass of débris and a larger conglomerate corpus be formed, or they may grow by the accretion of concentric layers. Which of these methods is operative is probably the expression of the interfacial tension between the small corpus and the surrounding fluid and débris and dependent on the lipid content of both. This is a hypothesis, since no correlation can be established between the morphologically visible lipid and the growth of the corpus, but the known effects of lipid on surfaces and the known lipid content of these bodies render it likely.

In contrast to this simple and direct process of origin and growth, a type of corpus is observed with a central nidus which differs in density, structure or staining reactions from the more peripheral portions. This nidus is frequently a large droplet of sudanophilic lipid or a smaller agglutinated mass of dark brown pigment granules, entirely similar to those found in the basal portions of intact epithelial cells. The lipid may be isotropic or anisotropic. Concentric lines are rarely placed near the center. A corpus of this type probably represents the agglutination and inspissation of cellular débris about a central nidus and may be designated as an agglutinative corpus in contradistinction to the simple cellular corpus and the conglomerate corpus. All types may subsequently grow by accretion in the form of concentric layers. That all corpora are not of the central nidus agglutinative type is shown by the failure to find the nidus in serial sections.

A study of the most peripheral of the concentric layers throws additional light on the problem of the transformation of cells into the

18. Eastman, J. R.: J. A. M. A. **29**:158, 1897.

corporal matrix and growth. In figure 4 *C* the outside layer is clearly still cellular in character with cell walls and a few nuclei still visible. Rarely a bare nucleus does not degenerate and may be embedded in the deeper layers. With this view the concentric lines represent periods of decreased growth and the broad zones active growth. The conception of cyclic growth is supported also by the evidence of new corpora formed about portions of the fractured corpus shown in figure 2 *C* and discussed in an earlier paragraph.

THE STRUCTURE OF PROSTATIC CALCULI AND THEIR RELATION TO CORPORA AMYLACEA

Under the term "prostatic calculi" are included all bodies formed in the acini which contain inorganic elements. This excludes all areas of tissue calcification which are the result of inflammation, all calcified thrombi or phleboliths and the exogenous calculi. For practical purposes, the term "inorganic elements" is synonymous with "calcium phosphate and carbonate." The demonstration and relative quantities of these substances will be considered in the chemical portion of this report. With this definition the group includes bodies from 100 microns in diameter to large irregular masses weighing 100 Gm., as reported by Podlasky.[2] The smaller examples may be studied in section and all transitions observed between the usual corpus and local deposits of calcium in the form of calcospherites similar to those deposited in cartilage. The calcium salts are deposited in an area of the corpus which shows no secondary changes, and it seems improbable that the original constituents enter into the formation of the calcium phosphate and carbonate. It is more likely that this is an infiltration similar to the deposition in osteoid tissue. The calcospherites gradually coalesce and form a solid mass of calcium salts. The calcium salts have no effect on polarized light, but when the calculi are stained with alizarin the typical crystalline doubly refractile calcium salt alizarin sulfonate appears. In addition to the chemical evidence to be submitted later, the positive von Kossa stain is accepted as histochemical evidence for the phosphate radical, and the formation of gas bubbles on treatment with dilute mineral acids, for the carbonate radical. Almost without exception there is a small amount of iron in the form of granules associated with the calcium deposit. Very rarely the calcium phosphate is deposited in the form of white or gray doubly refractile crystals. When these calcified masses are decalcified, the original matrix with concentric lines is visible.

If the larger calculi are decalcified, embedded in gelatin and cut in frozen sections, a concentrically laminated architecture is visible. In addition, a variable number of smaller corpora are trapped in the layers. Organic phosphorus can be demonstrated by the MacCallum technic in

this matrix as in that of the corpora. In addition to this similarity of structure, after removal of the calcium salts from the calculi, a mixed structure is observed in many instances: Frequently the small corpus shows a calcified center and a typical peripheral layer of matrix, or the reverse, and rarely is it possible to demonstrate an outer uncalcified layer on the large calculus.

Rarely the larger calculi, but frequently the smaller ones (single calcified corpora), are isolated in the stroma. No fibrosis or cellular infiltration results. The isolation of a corpus does not condition calcification since a corpus with calcospherites (early calcification) has never been observed free in the stroma.

On the basis of this evidence it may be said that the basic composition and the architecture of the matrix of the corpora amylacea and calculi are the same and that the only essential difference is one of size and infiltration of calcium salts.

ETIOLOGY

With the point of view of the pathogenesis of the corpora amylacea which has been developed in this paper, it is hardly possible to discuss etiology, since corpora are formed from desquamated cells and secretion and since desquamation and secretion are physiologic functions of the prostate. The chief problem is an explanation of the distribution according to age. Corpora are found in the prostate of the fetus at 7 months, but they are most abundant after 50 years of age. They are not abundant in acini with tall columnar epithelium. This is especially well seen in the tall hyperplastic epithelium of the prostate showing benign enlargement. There is a gradual transition to a lower, more cuboidal epithelium at about 50 years of age and this epithelium is probably more readily desquamated than the tall columnar type. The reason for this change of cell type and greater lability to desquamation probably lies in the changed endocrine relations at this period. That age alone is not an important factor is shown in a few prostates from persons 60 or even 70 years of age, in which the acini are lined with tall epithelium and the lumens contain very few corpora. The cuboidal and flat epithelial cells contain numerous pigment granules which may serve as a nidus. Also, as pointed out by Plenge,[19] doubly refractile lipid is more abundant in the senile prostate, and this, again, may influence the formation of corpora. In association with this change in the epithelial cells there is an easily demonstrable senile fibrosis of the stroma as well as a relative decrease in the smooth muscle tissue. The factor of fibrosis would cause partial obstruction of the ducts and consequent stagnation. With the advent of senile atrophy of the parenchyma this ductal obstruc-

19. Plenge, C.: Virchows Arch. f. path. Anat. **253**:665, 1924.

tion would be exaggerated. Assuming that the prostate contracts and empties its content, the decrease and cessation of active sexual life would contribute to stagnation. In addition to this physiologic factor there is the morphologic evidence of a decrease in the smooth muscle tissue of the stroma. Finally there is the usual thrombosis of the prostatic plexus with resultant passive hyperemia of the prostate and, as in any glandular organ, passive hyperemia causes increased desquamation.

In summary, the factors which favor the formation of corpora amylacea in the presenile and senile prostate are: change in the epithelial cell type and increase in the lability to desquamation, probably the result of changed endocrine relations; fibrosis of the stroma and senile atrophy of the parenchyma with partial duct occlusion and stagnation; decrease in the physiologic discharge of prostatic secretion and decrease in the content of smooth muscle, and increase of desquamation as the result of chronic passive hyperemia induced by venous thrombosis.

With the evidence that secondary softening and dissolution, probably the result of the action of the nuclease enzymes, may occur, the possibility that the senile prostate is deficient in these enzymes was suggested. As will be shown, a saline extract of the senile prostate with many corpora is just as active as that from the prostate of a middle-aged person in the alteration of the optical rotation of nucleic acid solutions. Hence, a failure to dissolve in senility cannot be offered as the explanation of the distribution of corpora according to age.

Throughout the urologic literature there has been an attempt to relate chronic infections to the formation of calculi and the occurrence of corpora amylacea. In the case of corpora, this study does not support the idea that infection has any direct relation. This is based on the experience that corpora are not found in glands the seat of so-called chronic prostatitis in any greater abundance or frequency than in normal glands. In the case of calculi, the problem is slightly different. The physiologic factors responsible for the formation of corpora do not include the infiltration of these bodies with calcium salts. The calculi are usually found in large cystic spaces embedded in yellow cheesy material, and gross dissection shows that one of the calculi occludes a duct. Microscopically the stroma about the dilated acinus or duct is infiltrated with lymphocytes. However, any acinus, with or without calculi, which contains retained secretion rich in lipids shows this periacinar lymphocytic infiltration. It has not been possible to stain bacteria in sections of these acini. Therefore, there is some doubt whether the infection and lymphocytic infiltration are primary, with secondary formation of calculi and dilatation, or whether the obstruction, dilatation and formation of calculi are primary, with the morphologic evidences of inflammation secondary to the retention of secretion. The absence of demonstrable bacteria in the presence of chronic infection is not of great

value. As mentioned in a previous paragraph, uncalcified corpora are not infrequently observed embedded in the inflammatory exudate within an acinar lumen. Although no alternative explanation for the infiltration with calcium salts and formation of large calculi can be offered, the evidence for a causal relation to chronic infection cannot be considered as well established. The surgical literature on calculi has been well reviewed by Kretschmer [20] and Gläsel.[21]

SUMMARY

Prostatic corpora amylacea are formed from desquamated epithelial cells and prostatic secretion. Some are formed by direct transformation of a single cell into a single corpus, while others are formed by conglomeration of the smaller corpora or by agglutination and inspissation of a large mass of degenerated desquamated cells.

Cyclic growth occurs by the addition of concentric layers and lines. Continued growth in a limited space results in faceted surfaces. Movement and rotation may occur between cycles of growth.

The effect on polarized light and the radial striae are due to the presence of one or more crystalline purines (adenine, xanthine and hypoxanthine).

Secondary central softening with fracture of the peripheral layers occurs. A fractural segment may serve as a nidus for a new corpus.

The increased incidence of corpora in senility is the result of a variety of factors which act through increased epithelial desquamation and stagnation in the ductal system.

Calcium salts may be deposited in a corpus and the continued growth result in a large calculus. The evidence that calculi are directly related to infection is not well established.

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20. Kretschmer, H. L.: Surg., Gynec. & Obst. **26**:70, 1918; **44**:163, 1927.
21. Gläsel, F. A.: Ztschr. f. urol. Chir. **2**:353, 1914.

CHEMICAL COMPOSITION OF PROSTATIC CORPORA AMYLACEA AND CALCULI

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In connection with the morphologic study set forth in the previous paper,[1] it was desirable to secure independent and related chemical observations. The observations have been divided into sections on inorganic substances, lipids, carbohydrates and proteins. The corpora were secured by gross dissection of formaldehyde-fixed or fresh prostates, washing in water until the corpora were free from secretion, tissue and fixatives, and drying at 20 C. All determinations and observations recorded have been repeated once or twice.

INORGANIC SUBSTANCES

Because of the practical impossibility of removing the water adherent to the surface of the corpora without loss of the water which is a part of them, a determination of the loss in weight on simple desiccation was not considered of value. However, a determination of the water lost by heating at 110 C. was made.

Sample 1, fresh unfixed corpora, was desiccated at 20 C. over sulfuric acid. It was then heated at 110 C. for five hours in an electric oven. The initial weight before heating was 15.6 mg., and the weight after heat desiccation, 15 mg. This gives a loss of 0.6 mg., or 3.3 per cent of the original weight. A duplicate determination gave 3.5 per cent.

Because of the assumption that corpora amylacea contain a substance identical with or similar to amyloid, a determination of the sulfur con-

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This investigation was suggested by Prof. J. Erdheim while one of us (R. A. M.) was a National Research Fellow in Medicine in Vienna. The material was collected in Vienna. Technical assistance was furnished by the Committee on Grants-in-Aid of the National Research Council. The chemical analyses were carried out in the Institute of Pathology of Western Reserve University.

1. Moore, R. A.: Arch. Path. **22**:24, 1936.

tent is of great interest. According to Wells,[2] some investigators have denied that pure amyloid contains sulfur, but the preponderance of evidence favors the view that amyloid contains organically bound sulfur. Wells [2] further stated that amyloid is soluble in the same general solvents as nucleic acid.

Sample 2, 226 mg. of air-dried corpora, was extracted with an alkaline solution of 1.5 per cent sodium hydroxide and 10 per cent sodium acetate. The extract was divided into two parts; one was acidified and made strongly acid with nitrohydrochloric acid, and the other was left untreated. The two were placed in an oven at 110 C. and evaporated to dryness. To the acidified extract additional quantities of nitrohydrochloric acid were added until the residue was pure white. The residues were taken up in hot water and the sulfate precipitated with barium chloride. Standards were prepared from standard sulfuric acid and comparisons made in the nephelometer. From the unhydrolyzed specimen a quantity of other material was precipitated by the barium chloride. The precipitate was collected and washed by centrifugation until the washings were free from barium. The residue was hydrolyzed with 1 cc. of nitrohydrochloric acid and the resultant suspension of barium sulfate compared with a standard similarly treated.

This method was expected to give figures for the total sulfur and the inorganic sulfur. The results were 0.852 mg. of total sulfur (as sulfur) and 0.617 mg. of inorganic sulfur and by difference 0.235 mg. of organic sulfur. This gives 0.1 per cent by weight of the original corpora as organic sulfur. On the assumption that about 3 per cent of the amyloid molecule is sulfur, there is about 7 mg. of amyloid in 226 mg. of corpora, an amount which clearly eliminates this substance from consideration as an important component of corpora amylacea if the basic assumption that amyloid is a sulfur compound is true.

The only other inorganic constituents considered of importance were the calcium and the phosphorus in the larger calculi.

Sample 3, 37.2 mg. of calculi, was treated according to the method of Kramer and Tisdall [3] for the determination of blood calcium. Calculated as calcium, 50.1 per cent of the calculi was calcium phosphate, $Ca_3(PO_4)_2$.

Sample 4, 52.3 mg. of calculi, was treated according to the method of Youngburg and Youngburg [4] for the determination of blood phosphorus. Calculated as phosphorus, 49.5 per cent of the calculi was calcium phosphate, $Ca_3(PO_4)_2$.

Other determinations varied between 45 and 60 per cent, calculated as calcium phosphate, $Ca_3(PO_4)_2$.

The evolution of gas on treatment of the calculi with dilute mineral acids demonstrated the presence of carbonates. No quantitative determinations were made. However, the deduction of the organic phos-

2. Wells, H. G.: Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925.

3. Kramer, B., and Tisdall, F. F.: J. Biol. Chem. **47**:475, 1921.

4. Youngburg, G. E., and Youngburg, M. V.: J. Lab. & Clin. Med. **16**:158, 1930.

phorus from the total would leave not over 5 per cent of the calcium available as calcium carbonate even with the known excessive calcium content of so-called tri-calcium phosphate,[5] $Ca_3(PO_4)_2$.

Finally there is the determination of the ash from corpora.

Air-dried corpora and calculi were ashed in an electric muffle-furnace at a dull red heat (about 500 C.) for six hours. The ash content of corpora varied from 50 to 60 per cent (52.6, 58.1 and 58.7 per cent), while that of calculi varied from 70 to 85 per cent (71.7, 75.4 and 83.2 per cent).

LIPIDS

Because of the known presence of phospholipids in the prostatic secretion, it has been assumed that corpora amylacea are composed of a substratum of these substances. Fürbringer[6] in an early paper concluded that corpora were composed essentially of lecithin. Björling[7] and Posner[8] suggested that the lecithin united with protein from degenerated cells and secretion to form a lecithalbumin. Castano[9] investigated corpora in the utricle and prostate and concluded that cholesterol is an important constituent. Joly[10] in his textbook stated that corpora contain a fair amount of lecithin while Young and Davis[11] mentioned cholesterol as a prominent component. MacCallum, as reported by MacKenzie and Seng,[12] found 46.7 per cent organic matter in large calculi and stated that this consisted in part of organic phosphorus compounds such as lecithin.

In this investigation microchemical and histochemical analyses of the corpora and of prostatic tissues and secretion have been made.

Microchemical Analysis of the Corpora for Lipids.—Corpora were extracted with alcohol and ether (3:1) and with chloroform. The phosphorus determinations were made according to the method of Youngburg and Youngburg.[4] Blank determinations never gave a value above 0.003 mg.

Sample 5, 64.4 mg. of air-dried corpora, was divided into sample 5a, 27 mg., and sample 5b, 37.4 mg. Aliquots yielded 0.0066 mg. of phosphorus in sample 5a

5. Bogert, L. J., and Hastings, A. B.: J. Biol. Chem. **94**:473, 1931.
6. Fürbringer, P.: Ztschr. f. klin. Med. **3**:287, 1881; Ztschr. f. Urol. **5**:169, 1911.
7. Björling, E.: Ztschr. f. Urol. **6**:30, 1912.
8. Posner, H. L.: Ztschr. f. Urol. **5**:722, 1911.
9. Castano, E.: Rev. de especialid. **2**:1007, 1927; abstr., Ztschr. f. urol. Chir. **25**:299, 1928.
10. Joly, J. S.: Stone and Calculous Disease of the Urinary Organs, St. Louis, C. V. Mosby Company, 1929.
11. Young, H., and Davis, D. M.: Young's Practice of Urology, Philadelphia, W. B. Saunders Company, 1926.
12. MacKenzie, D. W., and Seng, M. I.: J. Urol. **12**:243, 1924.

and 0.112 mg. of nitrogen in sample 5b. On the assumption that the two fatty acids in lecithin are palmitic acid, 0.0066 mg. of phosphorus equals 0.17 mg. of di-palmitic lecithin, which is 0.63 per cent of the dry weight of the corpora. After deduction of the nitrogen to satisfy this quantity of lecithin, there remains 0.0778 mg. unaccounted for. It is probable that this represents other nitrogenous bases, as choline and carnithine.

Sample 6, 21.1 mg. of air-dried corpora, was extracted with boiling chloroform. Tests for phosphorus on aliquots were negative. An aliquot (one fifth) when subjected to the Liebermann-Burchard test [13] gave a very faint green color, impossible to compare in the colorimeter. It approximates a standard containing 0.01 mg. of pure cholesterol; this is about 0.25 per cent of the dry weight of the corpora.

It is clear from these quasiquantitative determinations that a free phospholipid is present in amounts not over 1 per cent in corpora. The attempt to identify this phospholipid has been in part successful.

The alcohol and ether extracts from sample 5 not used for the phosphorus and nitrogen determinations were evaporated and taken up in acidified absolute alcohol. To one aliquot (one fourth as 5 cc.) 1 cc. of 1 per cent platinic chloride, and to another 1 cc. of 3 per cent gold chloride, was added. No crystals of the lecithin metallic salts formed after several days in the ice chest. To the remaining 10 cc. were added 2 cc. of saturated alcoholic solution of cadmium chloride and 3 cc. of distilled water. The crystals which separated (fig. 1 *A*) were similar to those prepared by a similar procedure with a lecithin solution. The crystals were suspended in alcohol and decomposed with hydrogen sulfide, and the supernatant fluid was evaporated in a hanging drop slide. The spherocrystals (fig. 1 *B*) melted between 38 and 45 C., and on cooling there was a sharp transition at 39 C.

The absence of crystal formation with the gold and platinum salts excludes lecithin in any more than minute amounts, while the formation of crystals with cadmium and the subsequently determined melting point of the phospholipid are a probable identification of sphingomyelin.

It seemed possible that lecithin was bound to a protein and would not dissolve in boiling alcohol and ether.

The chloroform-insoluble material from sample 6 was extracted for five hours with 5 per cent hydrochloric acid in 70 per cent alcohol. An aliquot (one fifth as 5 cc.) gave 0.455 mg. of nitrogen calculated for the whole, and another aliquot, 0.254 mg. of phosphorus. An attempt to isolate choline platinic chloride from an aliquot failed. By actual test with pure choline hydrochloride 1 mg. of choline could be identified by the method and in the quantities used. In a final aliquot no fatty acids could be isolated with sodium ethylate.

The foregoing observations show clearly that acid hydrolysis of corpora releases relatively large quantities of phosphorus, but with this hydrolysis no detectable quantity of choline or of fatty acids is liberated.

It may therefore be concluded that lecithin, free or bound, is not present in any appreciable quantity in corpora and that the small quantity of phosphorus soluble in alcohol and ether is probably sphingomye-

13. Liebermann, L.: Arch. f. path. Anat. u. Physiol. **54**:573, 1893.

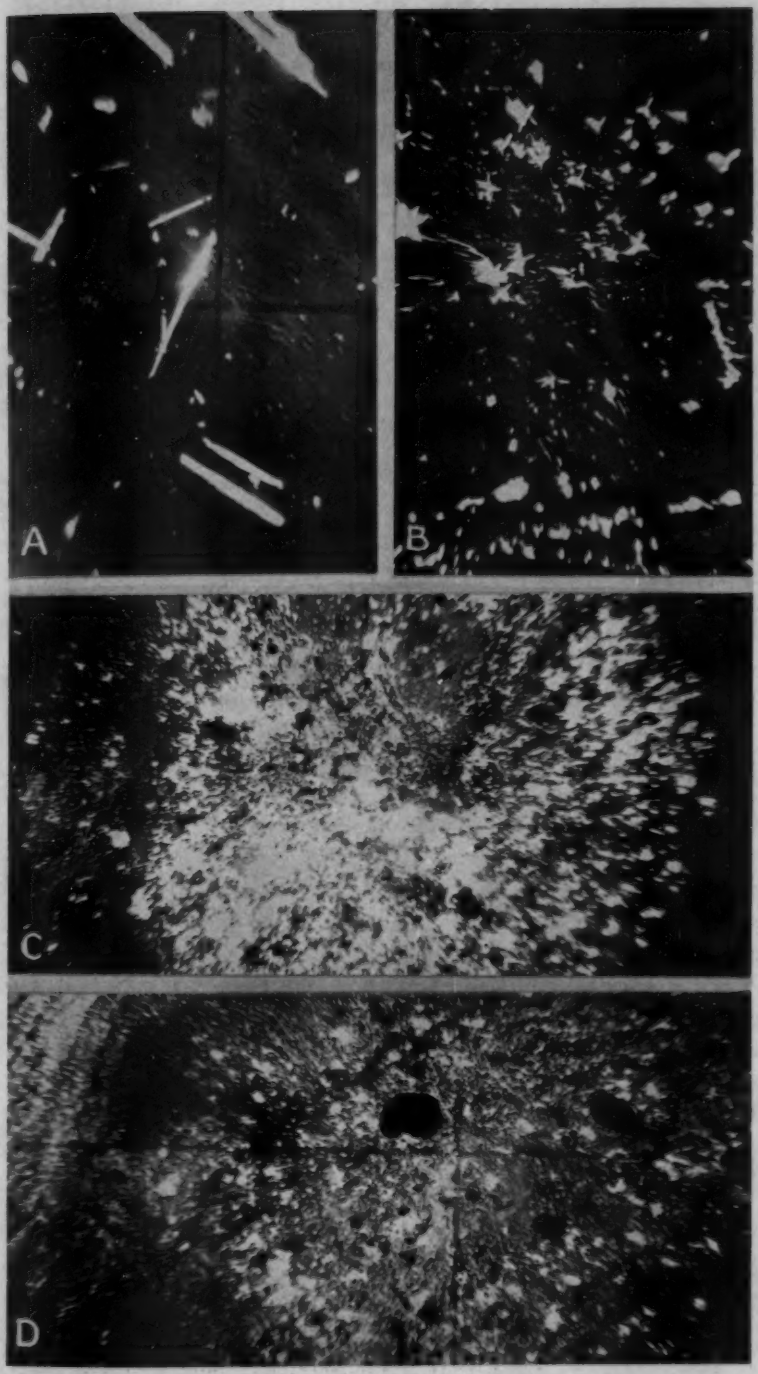

Fig. 1.—Crystals observed in corpora amylacea with polarized light and crossed Nicol prisms: *A*, crystals of lecithin cadmium chloride isolated from the alcohol-ether extract of corpora amylacea; × 140. *B*, liquid crystals of sphingomyelin isolated from the alcohol-ether extract of corpora amylacea; × 170. *C*, anisotropic crystals in an acinus with inspissated retained secretion; frozen section mounted in glycerin jelly; × 90. *D*, the area shown in figure 1 *C* after the section had been washed in acetone; × 90.

lin. This sphingomyelin may be associated with a small quantity of cerebrosides, but all attempts to identify a reducing substance in hydrolytic products of lipid extracts gave negative results.

Several samples of calculi have been subjected to analyses identical with those outlined for corpora, with essentially the same results. A very weak Pettenkofer reaction was secured on one chloroform extract of calculi, indicative of the presence of cerebrosides. About 0.2 mg. of cholesterol was found in 127 mg. of calculi. A pyridine extract gave about 1 degree of dextrorotation but no absorption bands in the visible spectrum.

Chemical Separation of Lipids in Prostatic Tissue.—The prostate of a 56 year old man removed three hours after death, that of a 42 year old man taken one and one-half hours after death and that of a 51 year old man taken two hours after death were separately freed from visible fat, minced, soaked in acetone for twenty-four hours in the icebox and dried in a current of air. The general scheme of separation of the phospholipids given by Maclean [14] was followed. The only substances identified were cephalin and sphingomyelin. Lecithin and the cerebrosides could not be isolated.

Histochemical Observation of Lipids in the Corpora.—Since the corpora amylacea are formed in the glandular acini of the prostate, admixture with prostatic secretion is probable, and a study of the lipids of the secretion should yield indirect evidence. The literature on the corpora amylacea is interwoven with that on prostatic secretion, largely owing to the early observations that double refractile lipid occurs in the secretion and that many of the corpora are doubly refractile (Posner [15] and Posner and Rapoport [16]).

Gelatin-embedded frozen sections of 118 prostates from persons from 2 days to 92 years of age were studied. Even cursory examination of the series showed that demonstrable lipid does not appear until the second decade of life. The observations are not sufficient to correlate definitely the occurrence of lipid with puberty, but this is highly probable, as pointed out by Plenge.[17] Plenge concluded that the lipid is most abundant between the ages of 30 and 50 and decreases after 60 years, and is the product of a true secretory activity. Kinoshita [18] came to the same conclusion, but he regarded the doubly refractile lipid as representing a decreased or abnormal activity of the cell and

14. Maclean, H.: Lecithin and Allied Substances: The Lipins, New York, Longmans, Green & Co., 1918.

15. Posner, C.: Ztschr. f. Urol. **5**:161, 1911; Berl. klin. Wchnschr. **46**:254, 1909.

16. Posner, C., and Rapoport, L.: Deutsche med. Wchnschr. **31**:492, 1905.

17. Plenge: Virchows Arch. f. path. Anat. **253**:665, 1924.

18. Kinoshita, M.: Ztschr. f. Urol. **14**:145, 1920.

found it in greater amounts after the age of 45 years. The observation that doubly refractile lipid in cells is more abundant after middle life is entirely consistent with these studies. In addition to cellular lipid, large collections occur in dilated acini with flattened epithelial cells, and the stroma shows a relative increase of connective tissue. It is probable that the entire process consists in a stenosis or occlusion of the duct with resultant stagnation of the secretion, a reabsorption of water and an apparent increase in lipids. The presence of these collections in senile prostates offers an opportunity to study the nature of the lipids.

Prostate 435, from a 54 year old man who died of pulmonary tuberculosis, contained numerous such collections. Figure 1 *C* shows a dilated acinus photographed with polarized light and crossed Nicol prisms. In addition to the material visible with this method there are globules of lipid which stain with sudan III and are red or rose-colored with nile blue sulfate. Treatment of such a section for twenty-four hours with cold acetone gives the picture shown in figure 1 *D*. About two thirds of the crystalline and all the noncrystalline lipid is acetone-soluble and hence not phospholipid. Similar treatment with alcohol or acetone followed by pyridine dissolves all but a very few crystals. The melting points and clearing points were observed with the usual apparatus and loss of double refraction accepted as the criterion (Chamot and Mason[19]). All but a few crystals melt below 75 C. A large part in untreated sections and almost all in acetone-treated sections lose their double refraction between 40 and 50 C. These crystals exhibit the property, mentioned in connection with sphingomyelin, that cooling results in many spherocrystals which change to irregular doubly refractile masses after from twelve to twenty-four hours. In untreated sections another large portion melts between 64 and 69 C., with a few melting around 60 and a few at about 71 C.

These observations demonstrate the probable presence of isotropic neutral fats and fatty acids and anisotropic lecithin and sphingomyelin.

Direct Histologic Investigation of the Corpora for Lipids.—Sections stained with nile blue sulfate show a variety of pictures. In general, the corpora stain different shades of blue with the darker parts in the center. There are occasionally concentric layers which stain light orange or red. Usually in the center but rarely in the peripheral layers there may be small orange, red or rose-colored droplets. These droplets are not doubly refractile, but the larger droplets may contain one or two small doubly refractile needle-like crystals. In corpora with vacuolated centers, the network stains greenish blue or pink or purple, and occa-

19. Chamot, E. M., and Mason, C. W.: Handbook of Chemical Microscopy, New York, John Wiley & Sons, Inc., 1931.

sional isolated nondoubly refractile orange or pink droplets are present. The radial lines in the centers of some corpora stain deep blue.

With dilute solutions of sudan III and scarlet red the droplets which were red or orange with nile blue sulfate are pink (series of five frozen sections each were prepared on six prostates and the five sections in each series stained, respectively, with nile blue sulfate, sudan III, alizarin, von Kossa's stain and prussian blue for iron). The inner portions of all corpora do not, except for occasional droplets, stain with sudan III, but in some instances the most peripheral layer and a rare intermediate layer of a corpus stain pink with concentrated solutions. The irregular homogeneous or finely granular corpora with concentric or radial lines at times stain a uniform pink. The concentric layers or entire corpora which stain pink bear no relationship to the double refraction which will be discussed in the next paragraph. The presence of a droplet of lipid in the center of the corpus in some instances raises the question as to whether or not every corpus contains such a droplet, which is not always visible because the section is not through the center. A series of ninety frozen sections of prostate 323 was prepared and twenty-five corpora followed. This series leaves no doubt that not every corpus contains a central droplet of lipid.

With osmic acid most, if not all, of the isotropic lipid and a portion of the crystalline lipid stains black. The latter is largely that part which melts between 40 and 50 C.

As pointed out, most investigators have correlated the lipid content with the observed double refraction of the entire corpus amylaceum. This double refraction [20] shows parallel extinction so that the polarization cross forms within each corpus (see fig. 1 *B* in article by Moore [1]). The polarization cross may be confined to the center or to the periphery or may be absent from certain concentric layers. Fresh corpora from unfixed material show this phenomenon, so it must be considered an essential part of the structure. Sections treated for forty-eight hours with absolute alcohol, 70 per cent alcohol, chloroform, acetone, ether, pyridine and acid alcohol retain this structure and determine the conclusion that the double refraction is not due to lipids. Ammonia, however, alters the corpus within a few hours so that the double refraction is entirely absent. This observation is recorded here to exclude lipids and will be used later to support the thesis that the crystalline purines determine double refraction.

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20. As reference works on the general problem of double refraction and liquid crystals we have consulted Schade (in Alexander, J.: Colloid Chemistry, New York, Chemical Catalog Company, 1928, vol. 2, p. 803). Prof. J. E. Hyde and Mr. S. A. Furcron of the department of geology, and Prof. H. S. Booth of the department of chemistry, of Western Reserve University aided us with advice on the use of the polarizing microscope.

Morphologic Observations on Lipids in the Calculi.—These observations were made by study of frozen sections of decalcified calculi and by study of ground sections embedded in Duco lacquer.

Calculi from prostate 648, from a 74 year old man who died of coronary sclerosis and cardiac infarction, were placed in 5 per cent

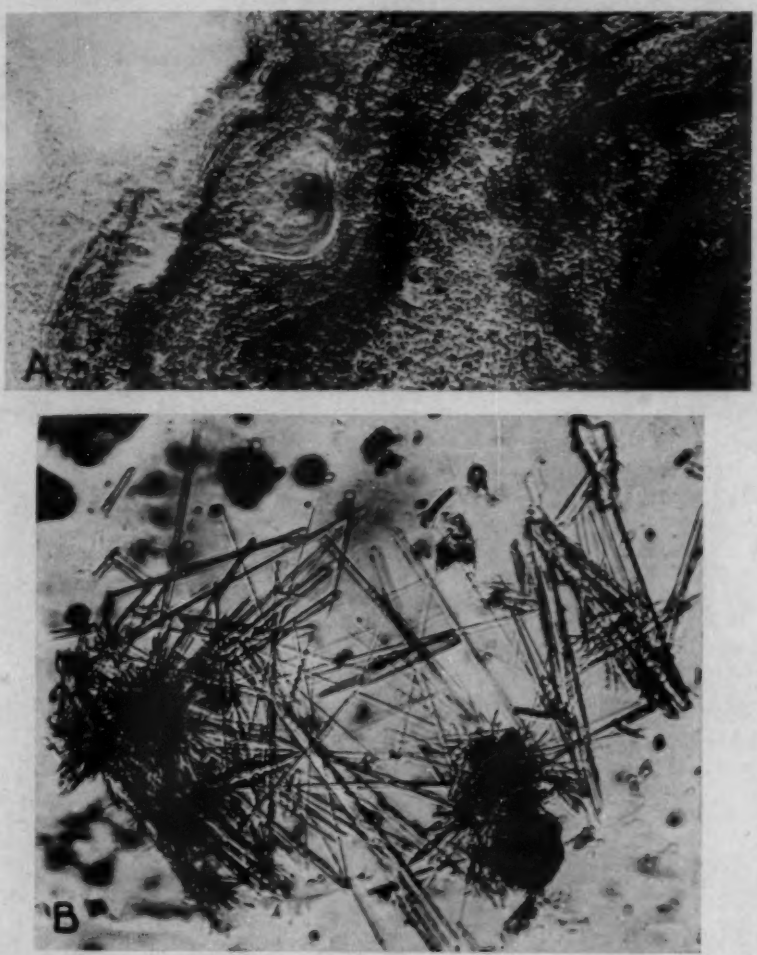

Fig. 2.—*A*, a corpus amylaceum trapped in the matrix of a large calculus; frozen section of a decalcified calculus; × 110. *B*, crystals of guanine hydrochloride isolated from corpora amylacea; × 80.

nitric acid for forty-eight hours, washed in water, embedded in 20 per cent gelatin and frozen sections prepared. These decalcified sections frequently show small corpora embedded in the layers of the calculus (fig. 2*A*). Sudan III stains show abundant small orange droplets

throughout all layers, and polarized light reveals only a very few doubly refractile crystals. All of this lipid is soluble in acetone and alcohol.

Ground sections [21] were prepared by taking a gross section of the calculus, grinding one surface smooth and embedding in Duco lacquer dissolved in acetone.[22] The section with polished surface down was mounted on a slide in thick Duco, dried and ground to the desired thickness.

Most calculi prepared in this manner show only a few doubly refractile spherocrystals, but three have been encountered which showed a large number. These crystals have a clearing point of about 50 C. and exhibit the same phenomenon noted before, that the cooled specimen shows many spherocrystals which gradually change over to irregular doubly refractile masses after from twelve to twenty-four hours. The fact that these preparations have been treated with acetone eliminates cholesterol and its esters as the source of this phenomenon.

CARBOHYDRATES

None of the tests for carbohydrates on alcohol-ether, acid and alkaline extracts has been positive. An alkaline extract of corpora consistently contains a reducing substance to the extent of approximately 1 per cent (calculated as dextrose) of the dry weight, but three attempts to isolate the osazone failed. A similar calculated amount of dextrose in the same volume gave typical osazone crystals. It is probable that this reducing substance is some nitrogenous base.

PROTEINS

From the morphologic evidence that corpora are formed from desquamated cells and secretion presented in the preceding paper it is logical to assume that proteins are an important constituent of these bodies. Preliminary chemical tests and the histochemical evidence of the presence of organic phosphorus and possibly of purine bases led to an approach to the problem through the group of nucleoproteins. The demonstration of xanthine and hypoxanthine in corpora from the prostate and utricle by Castano [9] also supported this view.

All preliminary extractions of the corpora were made with a boiling solution of 1.5 per cent sodium hydroxide and 10 per cent sodium acetate under a reflex condenser for one hour.

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21. Dr. T. J. Hill of the Institute of Pathology of Western Reserve University gave material aid in the preparation of the ground sections.

22. The Duco lacquer in acetone is in our experience preferable to shellac. Filtered shellac is not free from crystals since examination with polarized light shows many small curved doubly refractile needles, which melt at about 50 C. Shellac is dissolved in alcohol and hence would remove the phospholipids.

Sample 11, the extract of 56.2 mg. of dried corpora, was rendered acid with sulfuric acid, a sufficient excess being added to make 10 per cent, and hydrolyzed at the boiling point for one hour. The solution was neutralized to litmus and the phosphorus determined; 0.780 mg. was found. With a phosphorus factor of 10.8 for a tetra-purine nucleic acid, there was present 8.4 mg. of nucleic acid, which is 15 per cent of the dry weight.

However, it is still necessary to prove the existence of nucleic acid or its hydrolytic products since the aforementioned organic nonlipid phosphorus might be some other substance.

Sample 12, the extract of 11.4 mg. of dried corpora, was neutralized to litmus with acetic acid and then rendered just acid (1 per cent). The acidified extract was poured into 3 volumes of 95 per cent alcohol and the precipitate collected by centrifugation after forty-eight hours. After the residue had been washed once with 95 per cent alcohol, it was heated at the boiling point under a reflux for one hour with 10 per cent sulfuric acid. The hot acid extract was neutralized to litmus with ammonium hydroxide and sufficient additional hydroxide to make 2 per cent. The precipitate was washed once with 1 per cent ammonium hydroxide, redissolved in hot water with a few drops of sulfuric acid, and the color adsorbed on animal charcoal. The resultant colorless solution was treated at the boiling point with an excess of ammonia and the precipitate dissolved in 20 volumes of hot 5 per cent hydrochloric acid. After this solution had been in the icebox seventy-two hours the crystals shown in figure 2 *B* separated. Morphologically and in their reaction to polarized light they were identical with pure guanine hydrochloride isolated from pure sodium nucleinate.

The supernatant fluid from the first and second ammoniacal precipitations and the washing fluid were combined and rendered just acid to litmus with sulfuric acid. The purines were precipitated as the copper salt and decomposed with hydrogen sulfide.[23] The resultant solution was decolorized with animal charcoal, evaporated just to dryness and dissolved in 3 cc. of 5 per cent sulfuric acid. No crystals separated after one week in the icebox.

By these procedures an appreciable quantity of a chemical substance which behaves like nucleic acid and which yields definite crystals of guanine hydrochloride has been demonstrated. The inability to isolate adenine sulfate cannot be explained except on the basis of the small quantity present.

With slightly different methods of isolation it was thought that the isolation of other purines might be possible.

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23. In this procedure, for each 10 mg. of original corpora in a 5 cc. volume we added 2 cc. of 10 per cent copper sulfate and 2 cc. of 10 per cent sodium bisulfite. After the mixture had been heated for five minutes, successive 1 cc. portions of each salt were added at five minute intervals until insoluble red copper oxide appeared and then the preparation was given a final heating for five minutes. The copper salt of the purines with copper oxide was washed with hot water by centrifugation. The precipitate was suspended in hot water and hydrogen sulfide bubbled through the solution until all the precipitate was black (about thirty minutes). This was heated to boiling, 3 drops of 20 per cent sulfuric acid added to cause the copper sulfide to flocculate and the sulfuric acid solution of the purines separated by filtration.

The extract of sample 13, 10.6 mg. of air-dried corpora, was treated according to Rose [24] and Jones [25] for the isolation of guanine, adenine, xanthine and hypoxanthine from mixtures.

Again crystals of guanine hydrochloride were isolated without difficulty, but adenine picrate could not be demonstrated. Crystals of xanthine nitrate were recovered, but hypoxanthine nitrate did not crystallize.

With the existence of the purine bases in the hydrolytic products established by the isolation of crystalline xanthine nitrate and guanine hydrochloride, it was desirable to determine the amount of these substances, to serve as a check on the amount of organic phosphorus, and to determine whether or not this phosphorus was all bound as nucleic acid.

Sample 14, the extract of 37 mg. of air-dried corpora, was hydrolyzed with 10 per cent sulfuric acid, a few drops of acetic acid added and the whole rendered neutral to litmus with ammonia. The purines were precipitated as the copper salts and decomposed with hydrogen sulfide. The precipitation was repeated and the resultant copper salts of the purines used for a determination of nitrogen. Nitrogen to the amount of 0.088 mg. was found by the micro-Kjeldahl method. A similar determination on the combined washing and supernatant liquid gave 0.126 mg. of nitrogen.

These amounts of nitrogen calculated as purine yield 0.893 mg. on the assumption that guanine and adenine are present in equimolecular quantities, and this purine in turn equals 2.12 mg. of the di-nucleotide of adenine and guanidine. For a tetra-nucleotide this requires 1.95 mg. of the di-nucleotide of cytosine and thymine. Calculated as tetra-nucleic acid this is 4.07 mg., or 11 per cent of the dry weight. In all these quantitative determinations, the figures given are not accurate but serve more as a qualitative demonstration plus a very general quantitative record.

With this evidence it can be concluded that a nucleoprotein forms an important constituent of prostatic corpora amylacea. From the known property of nucleic acid to form a gel-like substance in concentrations of over 4 per cent and from the morphologic evidence that degenerated cells form the basis of corpora, it is a logical assumption that corpora possess their form and structure because of these substances.

With nucleoprotein and nucleic acid as important chemical constituents of corpora amylacea and with morphologic evidence of secondary softening of corpora, the demonstration of nuclease enzymes in the

24. Rose, W. C.: Physiol. Rev. 3:544, 1923.

25. Jones, W.: Nucleic Acids: Their Chemical Properties and Physiological Conduct, London, Longmans, Green & Co., 1920.

prostate became important. In this work the scientific papers of Levene and Medigreceanu [26] and Jones [27] and the monograph of Jones [25] have been followed.

Halves of the fresh prostates [28] from a 73 year old man, a 45 year old man and a 4 month old infant were each ground with 10 cc. of phosphate buffer solution of p_H 7 and sand in a mortar, a few cubic centimeters of toluene added and the preparation allowed to digest for twenty-four hours in the incubator at 38 C. The digest was filtered through hard filter paper and 1 cc. added to 24 cc. of a

Evidence of the Presence of Nuclease Enzymes in Corpora Amylacea

	Prostate					
		45 Years				
Time	73 Years	Fresh	Boiled	4 Months	Jejunum	Control
At once	0.37	0.51	0.82	0.53	0.45	1.05
1 hour	0.15	....	....	0.47	0.26	0.96
1½ hours	....	0.32	0.74	....	....	....
2½ hours	0.08	....	....	....	....	....
3 hours	....	....	....	0.43	0.18	1.05
5 hours	....	0.23	0.73	....	....	....
20 hours	....	....	....	....	0.11	....

0.25 per cent solution of sodium nucleinate in the same phosphate buffer. As a positive control a similarly prepared extract of the mucosa of the jejunum was used. As a negative control the sodium nucleinate solution alone was employed. All reagents and the polariscopic tubes were warmed to 37 C. and kept at this temperature except during the actual readings. Each reading, expressed in degrees of dextrorotation, is an average of five independent successive observations. As a control on the enzyme lability to heat, a portion of the extract of the prostate from the 45 year old man was boiled for five minutes and added to the sodium nucleinate solution. The separate observations were made at different times, each with a separate control, so that the figures are not comparable except in the vertical columns. The observations on the infant have been repeated twice.

The results shown in the accompanying table clearly indicate that an autolyzed extract of the adult prostate contains a heat-labile substance which rapidly alters the optical activity of sodium nucleinate. Further, this substance is not present in the immature prostate but is present in the senile prostate.

One gram of sodium nucleinate dissolved in 100 cc. of physiologic solution of sodium chloride and 8 cc. (representing about four tenths of the prostate) of a saline extract of the prostate of a 45 year old man were incubated with toluene for forty-eight hours. The resultant digest was treated according to Jones' [25] method for the isolation of guanine and adenine. Neither could be demonstrated. A part tested for phosphates gave a heavy precipitate of magnesium ammonium phosphate. Similar precipitation of an equal quantity of the organ extract gave only a few crystals.

26. Levene, P. A., and Medigreceanu, F.: J. Biol. Chem. **9**:375 and 389, 1911.
27. Jones, W.: J. Biol. Chem. **9**:129, 1911.
28. All extracts were made within two hours after death.

These two experiments demonstrate that the enzymatic hydrolysis of sodium nucleinate by extracts of the prostate liberates the phosphorus as inorganic precipitable phosphate and that the hydrolysis of purine proceeds beyond the primary stage. With this evidence it is very probable that the formation of crystalline purines and the secondary softening of corpora amylacea are the result of the action of nuclease enzymes. These enzymes are apparently related to the activity of the prostate, namely, secretion, since they were absent in extracts from the prostates of three children. The enzymes do not inhibit the formation or cause immediate and complete dissolution of all corpora because of the probable irregular presence of abundant secretion in the acini and other types of secondary changes, such as pigmentation, which alter the protein complex and render it indigestible.

SUMMARY

Prostatic corpora amylacea are composed largely of protein and nucleic acid, probably in the form of a nucleoprotein.

Secondary softening of corpora is brought about by the nuclease enzymes present in an actively secreting prostate.

The double refraction of corpora is due to crystalline purines and is not related to lipids.

Lipids are present in small quantities, but aside from their possible physicochemical properties they probably are not important constituents. The most important lipid in the prostate and its secretion is a sphingomyelin.

PROTECTIVE ACTION OF SULFHYDRYL AGAINST CARCINOGENESIS INDUCED WITH 1, 2, 5, 6-DI-BENZANTHRACENE

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The three properties of cells called proliferation, differentiation and organization, as well as others, are mutually dependent; nevertheless, they are distinct biologic functions and can be separated both by observation and by experiment. Other things being equal, it is fair to assume that the more cells there are in division the more chance there is for one or several to fail in subsequent differentiation and organization and to initiate a tumor. Since the sulfhydryl group increases the rate of cell division (but has only a secondary effect on the subsequent differentiation and organization [1]) and thus in any unit of time the skin of an animal treated with a compound containing sulfhydryl contains more cells in division than the skin of an animal used as a control, it might be expected that the simultaneous application of 1, 2, 5, 6-di-benzanthracene and a substance containing sulfhydryl to the skin of mice would result in a greater percentage of tumors and a shorter time for their development. On the contrary, the number of tumors was reduced and the time was lengthened.

EXPERIMENTAL INVESTIGATION

Procedure.—White mice were used, of a strain bred under uncontrolled conditions in this laboratory for four years. The incidence of spontaneous malignant growths was about 1 per cent, and in most instances the tumor was an adenocarcinoma of the breast. No instance of spontaneous carcinoma of the skin was observed.

Five variations in the experimental procedure were used, each with two hundred mice. The mortality incident to the application of the chemicals was higher in the mice treated with 1, 2, 5, 6-di-benzanthracene alone. The ranks were filled as the animals died, and the experiment was gradually tapered off. Observa-

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From the Lankenau Hospital Research Institute.

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1. Hammett, Frederick S.: Protoplasma **13**:331, 1931.

tions were made daily for twelve months. A few animals in which no tumor developed were treated longer, up to fifteen months.

The variations were as follows:

1. 1, 2, 5, 6-di-benzanthracene[2] in a 0.3 per cent solution in benzine was painted between the scapulae six days a week.

2. 1, 2, 5, 6-di-benzanthracene and para-thiocresol (0.5 per cent in hydrous wool fat) were applied on alternate days, six days a week.

3. Para-thiocresol was applied on alternate days for three weeks; then 1, 2, 5, 6-di-benzanthracene was added on the intervening days for three weeks; para-thiocresol alone was then employed on alternate days for three weeks, followed by the application of 1, 2, 5, 6-di-benzanthracene on the intervening days, and so on.

4. The same alternations were carried out, but the series was begun with 1, 2, 5, 6-di-benzanthracene.

5. Para-thiocresol was applied to the skin every other day for three weeks, then 1, 2, 5, 6-di-benzanthracene every other day for three weeks, then para-thiocresol and 1, 2, 5, 6-di-benzanthracene on alternate days, and so on.

Results of Experimental Procedures.—The following tabulation gives the results of the various procedures:

Procedure	Incidence of Tumor Percentage	Incidence of Carcinoma, Percentage
1	68	38
2	56	28
3	36	11
4	53	22
5	24	5

The word tumor as used in the tabulation designates both papilloma and squamous cell carcinoma. All the tumors were examined histologically, and invasion was used as the criterion by which they were classified as papilloma or carcinoma. The growths were divided arbitrarily into groups of small and large tumors. The length of time elapsing before well defined growth appeared is given in the following tabulation:

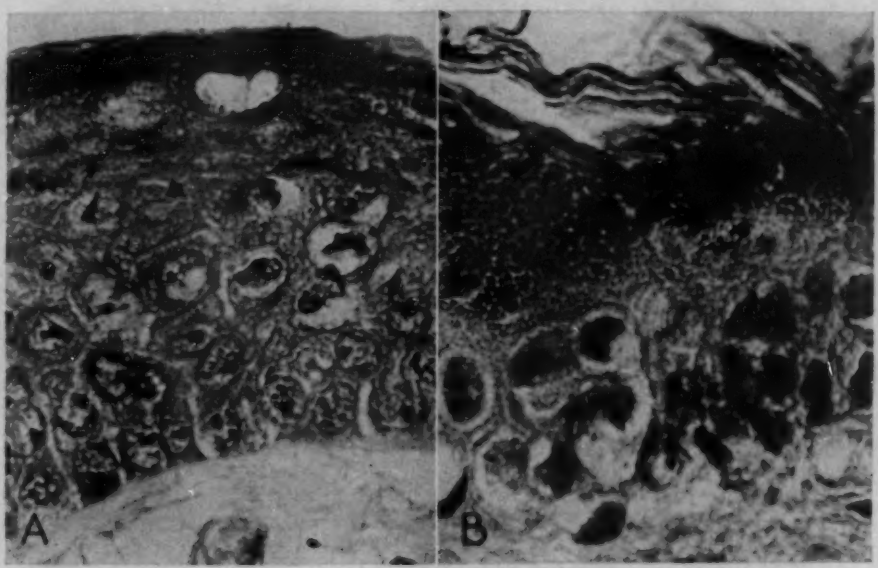
Procedure	Incidence of Large Growths, Percentage	Incidence of Small Growths, Percentage	Time Elapsing Until Growth Appeared, Days
1	83	17	244
2	56	44	268
3	46	54	270
4	58	42	227
5	33	67	297

From time to time sections of skin were removed for histologic examination from representative animals and finally from all the animals. The picture seen in the sections of skin treated with para-thiocresol alone was identical with that described in a previous article: There were more epithelial cells than in the normal skin; the various layers of the epiderm were defined much more clearly; the basement membrane was sharply defined, and there was no, or practically no, inflammatory reaction in the epiderm itself or in the underlying tissues.[3] The skin of the animals treated with 1, 2, 5, 6-di-benzanthracene alone was also thickened; there were also more cells than are usually present and the layers were better

2. 1, 2, 5, 6-di-benzanthracene was obtained from the Eastman Kodak Co., Rochester, N. Y.

3. Reimann, Stanley P.: Am. J. Cancer **15**:2149, 1931.

defined, but these changes were on a lesser scale than those in the skin treated with para-thiocresol. A distinct difference was detected in the organization. The cells of the basal layer tended to be oriented less regularly; there were more variation in the shape and size of the individual cells, more crowding and a less sharply defined basement membrane. The time element was about as follows: The sulfhydryl reaction was developed to its full extent in about three weeks when the material was applied three times a week and in about two weeks when applications were made daily. Hints of the forthcoming reaction, however, were manifest even twenty-four hours after the second application. Experience with mice (rats and guinea-pigs) for the past five years has shown that when hyperplasia and the succeeding enhancement of differentiation and organization of the cells so produced have reached their height no further progressive growth processes occur, but conditions are maintained *in statu quo*. It is significant that



Photomicrographs of sections of the skin of mice, showing (*A*) the sulfhydryl reaction, the criteria of which are regularity of the basal cells, a distinct basement membrane and enhanced differentiation of the spinous and pigment layers, and (*B*) the 1, 2, 5, 6-di-benzanthracene reaction, with irregularity of the cells of the basal layer, indistinct and irregular basement membrane and poor differentiation of cells in the spinous and pigment layers.

the organization remains orderly. When applications of a substance containing sulfhydryl were discontinued, the skin returned to normal in from three to four weeks and remained so. This therefore is probably the replacement time for the skin of the mouse and rat. In the skin of none of the many animals treated during recent years with a compound containing sulfhydryl has even papilloma appeared, no matter how long the applications were continued or how long the animals were kept after the applications. Only when an irritant was added were there abnormalities such as have been described.[3]

With 1, 2, 5, 6-di-benzanthracene alone changes were detected as early as with a substance containing sulfhydryl, but, of course, in many animals they went on to the production of tumor. The picture varied when both para-thiocresol and 1, 2, 5, 6-di-benzanthracene were used, depending in general on which substance was applied first. In an attempt to discover quantitative relations, the specimens of skin were compared in millimeter lengths and classified on the basis of the organization of the cells. The condition was called a sulfhydryl reaction when the arrangement was orderly (as shown in the photomicrograph) and a di-benzanthracene reaction when there was less diagrammatic organization. The results, rough as they are, indicate that in skins treated with a substance containing sulfhydryl there was a ratio of 9 mm. of orderly growth to 1 mm. of slightly irregular growth. In skin treated with 1, 2, 5, 6-di-benzanthracene there were 2 mm. of orderly growth and 8 mm. of irregular growth; in skins treated with both the proportions ranged between these values. The time element is important, as already stated, so comparisons were made of specimens with skin which had been treated for, respectively, three weeks and six weeks.

In the amount of keratinization differences were evident, more being produced in the skin treated with 1, 2, 5, 6-di-benzanthracene. As already stated, in few instances did the skin treated with a substance containing sulfhydryl show inflammatory reaction, and when present it was minimal; on the contrary, in most instances the skin treated with 1, 2, 5, 6-di-benzanthracene showed this reaction, beginning with a few plasma cells and wandering cells and slight prominence of vessels and progressing to frank inflammation in later specimens. When both para-thiocresol and 1, 2, 5, 6-di-benzanthracene were applied, the inflammation was also present. Obviously, mitosis was accelerated with applications of both substances, but counts failed to reveal significant difference in the number when specimens comparable in the time of treatment were chosen. The proliferation-accelerating action of 1, 2, 5, 6-di-benzanthracene was well shown in Obelia geniculata,[4] but the obvious additional effects on differentiation and organization were not demonstrated in this material. Perhaps its action as a stimulus to proliferation is indirect, through its effect on differentiation and organization, which it distorts from the very beginning. A natural initiator of proliferation is, of course, a break in differentiation and organization. Finally, it may be stated that attempts to guess the treatment accorded the skin by examination of the microscopic section resulted in a correct diagnosis in about 80 per cent of the instances when only one or the other chemical was involved and in about 60 per cent when both were used.

COMMENT

Berenblum [5] discovered that mustard gas, which is di-chloro-di-ethyl sulfide, inhibits the formation of tumor by tar and 1, 2, 5, 6-di-benzanthracene. In a further communication, he reported that some compounds chemically related to mustard gas also inhibited tumor formation but that others had no such effect. In addition, the chemically unrelated cantharidin had definite inhibitory properties. To the careful deductions derived from his experiments it is possible to add certain considerations based on the present experiments. The essential difference lies in the

4. Reimann, Stanley P., and Hammett, Frederick S.: Am. J. Cancer **23**:343, 1935.

5. Berenblum, I.: J. Path. & Bact. **32**:425, 1929; **34**:731, 1931; **40**:549, 1935.

fact that the sulfhydryl group is a naturally occurring chemical combination and that it is essential to proliferation, whereas the mustard gas and other compounds are foreign.

Berenblum was interested in the association of irritation and anticarcinogenic action. He could not decide whether irritation is requisite and suggested that it probably plays an essential rôle. Perhaps it must be of a particular kind to prevent carcinogenesis. Sulfhydryl is not an irritant, in contradistinction to mustard gas; at least if it is, there is no anatomic evidence in the experience of the last five years. Apparently, then, the anticarcinogenic activity of sulfhydryl is not due to irritation. Was it fortuitous in Berenblum's experiments?

Berenblum in 1929 expressed the opinion that mustard gas acts on the animal, i. e., on its skin, and his further experiments bore out this conclusion. We are of the same opinion; that is, the chemicals do not directly neutralize each other. Granted that this is true, it is possible to present a working hypothesis in which this and other observations are correlated. This must be put into biologic terms, since chemical data are wanting, but the results, nevertheless, present several points of departure for future experiments.

An undifferentiated cell contains more potencies than will be expressed. Even when these potencies are restricted, such as those of a basal cell of the skin, at least two are present, as demonstrated anatomically by the cells. When the environment determines which of the two potencies is to be expressed, this determination takes place without anatomic change that can be detected by present methods. In other words, it is not possible by looking at a cell which appears undifferentiated to tell whether or not its particular path of differentiation has been determined. After a variable lag time the cell proceeds on its way to differentiation, which, chemically speaking, means that the internal make-up of the cell is rearranged, with or without the addition of material from its immediate environment. (This statement can be made irrespective of the merits of the theories either of the independent or of the dependent differentiation of the mechanism.[6]) When a substance containing sulfhydryl is applied to the skin, the rate of cell multiplication is accelerated directly, and this is followed by the indirect effect, viz., increase in the rate and degree of differentiation and organization. It is highly improbable that dedifferentiation of a cell occurs; i. e., when once the mechanism for differentiation is set into activity, it proceeds without retracing its steps. There is no way of telling when determination takes place within a cell. Since the rate and degree of differentiation and organization are accelerated by accelerating cell proliferation,[1] it is justifiable to assume that determination also is

6. Weiss, Paul: Physiol. Rev. **15**:639, 1935.

accelerated. Thus, if determination takes place promptly after division in many cells, the conclusion is reached that when a tumor is produced in the skin of an animal rubbed with both 1, 2, 5, 6-di-benzanthracene and a substance containing sulfhydryl the former substance must exert its disorganizing effect between the end of division of a cell and the instant of determination of its potency. In contrast to the action of sulfhydryl, the effect of 1, 2, 5, 6-di-benzanthracene is exerted not on division but on the subsequent differentiation and organization which are demonstrated in the sections. A tumor must come from and have cells capable of division. The experiments reported in this paper and those of Berenblum deal with the skin, and the differentiated cells of the skin can no longer divide. Theoretically, 1, 2, 5, 6-di-benzanthracene must exert its disorganizing effect on one or two or all three of the following possible stages: an undifferentiated cell, a cell the potency of which has just been determined or a cell which has just started on its path to differentiation. On the basis of considerations of the time element, already mentioned, the conclusion is reached that its effects are exerted on or in the undifferentiated cell before determination.

Other considerations lead to the same conclusion. From procedures 1 and 4 in the experiments described in this paper, it is obvious that when the cutaneous applications are begun with 1, 2, 5, 6-di-benzanthracene all the cells which are stimulated to divide (directly or indirectly, as already noted) are subjected to its disorganizing influence. When the applications are begun with a substance containing sulfhydryl (procedures 3 and 5), a number of cells are stimulated to division and to accelerated determination and differentiation, leaving fewer cells capable of being influenced by the subsequently applied 1, 2, 5, 6-di-benzanthracene. Thus, there should be less disorganization and fewer tumors, and these results were observed experimentally. Finally, it is well known that the application of carcinogenic agents, such as 1, 2, 5, 6-di-benzanthracene and aniline, as well as the presence of viruses, is followed by tumor formation, even when the applications have been discontinued for months (or years in human subjects with tumor arising from aniline substances). From our results it seems that the stage is set for subsequent tumor formation surely in the first three weeks of the application of 1, 2, 5, 6-di-benzanthracene and possibly earlier. It is significant that two or three weeks is required with either a compound containing sulfhydryl or 1, 2, 5, 6-di-benzanthracene for development of the full reaction and that from three to four weeks is needed for elimination of the hyperplasia induced with sulfhydryl. But if months can elapse between the cessation of the applications and the beginning of tumor formation, the effect of carcinogenic agents must be produced on or in cells of the skin which are capable of further differentiation and

at a time before their differentiation is determined. For if the cells are differentiated, or on the way to it, they can no longer divide and thus are incapable of initiating a tumor. In such cases, therefore, the damage must be done to a cell capable of division, and, furthermore, this injury must be of such a nature that it is transmissible from the mother cell to an indefinite number of daughter cells. This is called somatic mutation.

The definition of neoplasm, as proposed in previous communications,[7] is strengthened by these experiments.

SUMMARY

The carcinogenic agent 1, 2, 5, 6-di-benzanthracene and a substance containing the proliferation-stimulating group sulfhydryl, para-thiocresol, were applied to the skin of mice in various combinations. The incidence of cutaneous tumor was reduced by the action of sulfhydryl. The anticarcinogenic effect of sulfhydryl was exerted by way of the animal, i.e., its skin; irritation played no part in the protection. In the discussion the three separable properties of cells, viz., proliferation, differentiation and organization, are invoked for clarity of exposition. Direct, indirect and circumstantial evidence is adduced to support the following propositions: Stimulation of the rate of cell proliferation alone does not lead to neoplasia; carcinogenesis is caused by damage to the potencies of differentiation and organization; this damage must be transmitted by a cell capable of division, or by somatic mutation; when a cell reaches a certain degree of differentiation it can no longer divide and the damage to the potencies of differentiation and organization must occur before potency is determined, at least in cutaneous cells.

The previously enunciated definition of tumor is strengthened.

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7. Reimann, Stanley P.: Arch. Path. **15**:675, 1933. Reimann, Stanley P., and Hankele, Ethel Rahe: Arch. Path. **17**:764, 1934.

BRONCHOPULMONARY MONILIASIS

ITS RELATION TO OBSCURE CHRONIC PULMONARY INFECTION

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Bronchopulmonary moniliasis is here defined as an infection of the lungs in which a pathogenic species of Monilia (Monilia albicans) appears to play an important etiologic rôle in the production of structural lesions. It is essentially a chronic and progressive disease and presents a characteristic clinical syndrome, on which the diagnosis largely depends.

The condition, originally considered as a tropical or subtropical disease, was first observed in 1905 by Castellani [1] among the tea workers of Ceylon, who evidently contract the infection through the inhalation of tea dust contaminated with the organism. Numerous reports, mostly clinical, have since been made from various parts of the world. The first case to be recorded in this country was described in 1915 by Boggs and Pincoffs,[2] who contributed a complete necropsy report, one of the few that are available on the subject. The condition rarely terminates fatally; therefore, the diagnosis in the majority of instances is made on the basis of clinical observation supported only by laboratory data.

CLINICAL SYMPTOMS

The clinical symptoms of bronchopulmonary moniliasis have been described by Castellani,[1] Joekes and Simpson,[3] Johns,[4] Stovall,[5] Warr,[6] the Flinns [7] and others. Three clinical forms are generally described—the mild, the moderate and the severe.

The mild form of the disorder is characterized by a slight but persistent cough which may last for weeks. The temperature is not

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1. Castellani, A.: Fungi and Fungous Diseases, Chicago, American Medical Association, 1927, p. 121.
2. Boggs, Thomas R., and Pincoffs, M. C.: Bull. Johns Hopkins Hosp. **26**: 407, 1915.
3. Joekes, T., and Simpson, R. H.: Lancet **2**:108, 1923.
4. Johns, F. M.: New Orleans M. & S. J. **77**:8, 1924.
5. Stovall, W. D., and Greeley, H. P.: J. A. M. A. **91**:1346, 1928.
6. Warr, Otis S.: Ann. Int. Med. **5**:307, 1931.
7. Flinn, John W.; Flinn, Robert S., and Flinn, Zebud M.: Ann. Int. Med. **9**:42, 1935.

elevated, and there is no appreciable physical sign of pulmonary disease. The sputum is scanty. A diagnosis of chronic bronchitis is usually made. Frequent recurrence of the symptoms is the rule.

The moderate form of the disorder presents symptoms and signs which are somewhat exaggerated. There may be a continued low grade fever. The cough is more troublesome; the sputum is mucopurulent and tenacious. Recurrence is common, or there may be continued activity of the symptoms with intervals of quiescence. A diagnosis of chronic bronchitis, bronchiectasis or bronchial asthma is usually made.

The severe form of the disorder presents two distinct clinical pictures. The patient suffering from the mild or moderate form of this condition may suddenly show signs of acute pneumonia (Tenney [8]) involving a wide area of one lung. This may simulate typical lobar pneumonia or bronchopneumonia or may represent a diffuse inflammation of the lung, in which pathogenic micro-organisms usually play the principal rôle. At the same time infection affords a fertile soil for the growth of the fungus. The patient is acutely ill, with elevation of the temperature and every evidence of acute pulmonary infection. This condition, lasting for a week or more, subsides completely or may be followed by any one of the complications of acute pneumonia. A history of previous attacks of "pneumonia" is usually elicited.

The second type of the severe form of this disease may result from a complication of the preceding type or may represent a progressive low grade infection of long standing in which no etiologic agent can be demonstrated save a pathogenic Monilia. The disease runs a prolonged and progressive course with periods of exacerbation of the symptoms. The temperature is hectic, and the patient complains of night sweats. There is gradually developing emaciation, and the patient loses weight and strength. He suffers from attacks of dyspnea and severe paroxysmal coughing, which are worse during the night. The sputum shows an increase in amount and is mucopurulent, tenacious, glairy and often hemorrhagic. It has been variously described as "curdy," "lumpy," "gruel-like" or "like cooked tapioca" in consistency and appearance. It may emit a yeasty or sweetish odor. It may become frankly purulent or copious and fetid owing to secondary bacterial invaders. The physical signs are those of patchy consolidation and fibrosis, of purulent bronchitis and bronchiectasis and, often, of abscesses and cavities, usually confined to the lower portions of the lungs. In short, the whole clinical picture is extremely difficult to distinguish from that of advanced pulmonary tuberculosis, with cavities in which Monilia is known to be a frequent secondary invader. In advanced stages a gradual embarrassment of the heart may develop owing to extensive

8. Tenney, C. F.: Internat. Clin. **3**:33, 1930.

fibrosis of the lung, resulting in decompensation and death from failure of the right side of the heart. This complication, while apparently not recorded by other authors, appears to be an important contributing cause of death in this disease (cases 3 and 4).

The roentgenograms of the lungs may show merely the exaggerated linear markings usually observed in cases of chronic bronchitis or bronchiectasis. During the acute febrile stage there may be a widespread shadow indicative of acute diffuse pneumonia, and a diagnosis of lobar pneumonia is usually made. In chronic advanced stages there may be seen irregular, mottled or feathery shadows, with peribronchial thickening and fibrosis and intervening areas of emphysema or bronchiectatic cavities in a large portion of the lungs. True cavities are occasionally present. The differentiation from chronic pulmonary tuberculosis is often difficult to make on the basis of roentgenograms.

LABORATORY DIAGNOSIS

The organism responsible for this condition is a fungus of the genus Monilia, belonging to the class of Hyphomycetes, or fungi imperfecti, and is characterized in situ by the presence of spores, budding forms and mycelial filaments. It is reproduced by free-borne spores, and it possesses no ascospores. It is one of the so-called yeastlike fungi and must be differentiated from the other members of the group.

While several species of the genus Monilia have been held responsible for infection of the respiratory tract, the present tendency seems to be to recognize only a single pathogenic form of this organism, namely, M. albicans, in this disease, as reported by Henrici,[9] Stovall,[10] Stone and Garrod[11] and others. However, the mere demonstration of a pathogenic Monilia (M. albicans) in the sputum does not establish the diagnosis of bronchopulmonary moniliasis.

The sputum must be obtained directly from the lungs, after every precaution has been taken to prevent contamination from other sources. The sputum often resembles typical "asthmatic" sputum in consistency and appearance. On close inspection, small cheesy granules may be observed, which represent minute masses of the fungus. The sputum persistently fails to show tubercle bacilli. Of the cellular elements, eosinophils may predominate. The yeastlike bodies, budding forms and sometimes branching filaments of Monilia are demonstrated with comparative ease, especially during the active stage of the disease. In exceptional cases, however, the organism may be demonstrated only by means of the proper cultural methods.

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9. Henrici, Arthur T.: Personal communication to the author.
10. Stovall, W. D., and Pessin, S. B.: Am. J. Clin. Path. **3**:347, 1933.
11. Stone, Kenneth, and Garrod, L. P.: J. Path. & Bact. **34**:429, 1931.

The identification of the isolated strain of Monilia on the basis of the biochemical reactions on carbohydrate mediums, a method originally employed by Castellani,[1] may be attempted. However, the more simplified technic of Stovall,[10] who proposed to recognize only three types of this organism, namely, M. albicans, which is pathogenic, Monilia candida and Monilia parapsilosis, appears to be sufficient. Wachowiak and his co-workers [12] concluded that the use of carbohydrate mediums for the differentiation of the species of this organism is not justified. Jacobson [13] summarized his conclusion as follows: "The difficulty lies not merely in terminology but also in the methods of identification since the organism may show a difference in morphologic behavior under different artificial conditions."

The differentiation of the species by agglutination, such as has been attempted by Benham,[14] Stovall [15] and others, and the complement fixation and precipitation methods in rabbits, reported by Stone and Garrod,[11] as routine clinical procedures seem impracticable.

Other methods of diagnosis have been attempted both clinically and experimentally, with results not sufficiently conclusive to justify a final evaluation. The intracutaneous injection of a suspension of living untreated organisms of Monilia pinoyi was reported as giving positive results in eighteen cases of bronchomoniliasis and negative results in fifty-three controls by Grossi and Balog,[16] who had failed in a similar attempt with the heat-killed organisms. Barnard [17] found this test accurate in 75 per cent of cases of bronchial asthma in which this fungus was isolated from the sputum. Various serologic reactions also have been used clinically with indifferent results by Warr,[6] the Flinns,[7] Hoffstadt and Lingenfelter [18] and others.

Animal inoculation should be employed in order to determine the pathogenicity of the strain of Monilia isolated. Castellani [1] stated as his opinion that the organism responsible for bronchomoniliasis not only must be pathogenic for laboratory animals but must be capable of causing pseudotuberculous nodular lesions in the lungs of the animals. He made a distinction between primary and secondary bronchomoniliasis, basing his diagnosis on whether or not the organism is capable of producing the pseudotuberculous nodules in the lungs. Such a dictum must be accepted with reservation.

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12. Wachowiak, M.; Marr, J.; Hagebusch, O. E.; Randall, W. A., and Fleisher, M. S.: J. Infect. Dis. **54**:35, 1934.
13. Jacobson, Harry P.: Fungous Diseases, Springfield, Ill., Charles C. Thomas, Publisher, 1932, p. 77.
14. Benham, Rhoda W.: J. Infect Dis. **49**:183, 1931.
15. Almon, L., and Stovall, W. D.: J. Infect. Dis. **55**:12, 1934.
16. Grossi, Gino, and Balog, Paul: J. Trop. Med. **32**:253, 1929.
17. Barnard, J. H.: M. Rec. **139**:534, 1934.
18. Hoffstadt, R. E., and Lingenfelter, J. S.: Am. J. Trop. Med. **9**:461, 1929.

MODE OF INFECTION

The mode of infection is a matter of conjecture. The fungus is extremely resistant to drying and to ordinary antiseptic measures. Two important factors must be recognized: First, direct inhalation of contaminated dust or air may cause the deposition of the fungus in the lower respiratory tract of man. The observation of Castellani,[1] who found this condition among the tea tasters and the coolies toiling in the dust of the tea factories of Ceylon is of significance. He [19] was able to produce the nodular lesions in the lungs of guinea-pigs by subjecting them to repeated insufflation of tea dust daily for eleven months. The organism responsible for clinical bronchomoniliasis has been isolated also from dried bird food,[20] dried fruit and straw.[21] Second, aspiration of the organism from the lesions in the upper air passages may cause their deposition in the lower bronchi and pulmonary alveoli. At least three of the five patients in my series were suffering from chronic infection of the upper air passages. In one of the three the organism was demonstrated in the hypertrophic mucosa of the antrum, which was removed at operation (case 5). Transmission of the organism from lesions elsewhere in the body also is thought to be a possibility.

REPORT OF CASES

Case 1.—A woman aged 38 was admitted to the hospital on Feb. 24, 1935, suffering from a severe cough with rusty sputum and pain in the left side of the chest, which began a few days before with a chill and a rise in temperature to 104 F. A diagnosis of lobar pneumonia was made. Two weeks prior to the onset of the present illness the patient had had a similar attack and had remained in bed for a week.

Examination showed a few moist râles and a friction rub in the lower left portion of the chest. The sputum was mucopurulent, copious and streaked with blood. Pneumococcus was isolated but was not of the known types. Masses of yeastlike bodies, budding forms and filaments, later identified as M. albicans, were readily demonstrated in the sputum. A blood count showed: hemoglobin content, 60 per cent; white cells, 11,500, and neutrophils, 87 per cent. Urinalysis gave negative results. A roentgenogram of the chest showed a wide area of consolidation in the lower portion of the left lung.

A definite consolidation of the lower portion of the left side of the chest developed, with physical signs of lobar pneumonia. The temperature ranged from 98.6 to 103.8 F., the pulse rate from 110 to 128 and the leukocyte count from 8,750 to 15,000 during the first five days; but normal levels were soon reached and remained during the rest of the patient's stay in the hospital, except that the pulse rate was always relatively rapid and there were a few occasions when the temperature rose to 101 F. The resolution was slow, although the patient was free from any evidence of complication or of continued intoxication. She perspired freely and was utterly exhausted after each attack of severe coughing.

19. Castellani, A.: Brit. M. J. 2:1208, 1912.
20. Mantner, H., cited by Warr.[6]
21. Grossi and Balog, cited by Warr.[6]

This was the outstanding symptom during the forty-five days of hospitalization. The cough was severe and came on in paroxysms. It became more troublesome during the night and early hours of the morning and could be heard from a distance as loud, strong whoops or barks. The expectoration was mucopurulent, at first hemorrhagic, extremely tenacious and indistinguishable from the sputum of a patient with typical bronchial asthma. It showed many eosinophils. During the height of the illness the organisms were abundant. Later, following medication, the sputum became more mucoid, and the organisms were demonstrable only in cultures.

The history of this patient revealed that multiple deforming arthritis developed in 1920. It started in the large toes and extended to practically all the joints of the body. The patient began to cough in the fall of 1931. At about the same time there was an abscess in the throat, with considerable discharge into the back of the throat. Numerous subcutaneous nodules were present, usually over the deformed joints, which became tender and inflamed and finally broke down, discharging a thick, tenacious, stringy, yellowish material. In the summer of 1932 the patient coughed almost constantly and raised large quantities of thick, stringy white substance which nearly choked her. Often the spasms of coughing started after slight exercise and lasted for an hour. The patient had difficulty in breathing. There was a wheezing sound, and a great deal of pain was felt in the chest. The patient was told that she had a nervous cough. During the winter of 1934 and 1935 she had frequent colds and an almost chronic sore throat. The temperature rose to 103 F. on several occasions. The patient had been a semi-invalid for several years.

About a year before the patient's present admission to the hospital one of the subcutaneous nodules was removed for examination and was found to be sterile. Histologically, it showed the structure of a typical rheumatic nodule. Two of these nodules broke down during the patient's present stay in the hospital. Culture of the pus from the discharging ulcer showed M. albicans.

Under iodide therapy the patient's condition rapidly improved. She has been entirely free from cough and expectoration and is now employed in a gainful occupation for the first time in several years. The subcutaneous nodules have ceased to discharge. Recently a small amount of tenacious sputum was obtained after forced expectoration (about a year after the onset of the last illness). M. albicans was isolated on culture. Many eosinophils were present in smears.

Comment.—This case is an instance of typical clinical bronchopulmonary moniliasis, closely resembling chronic bronchial asthma. Diffuse pneumonia (type I, the severe form) developed suddenly. The appearance of discharging subcutaneous nodules (from which M. albicans was isolated later) simultaneously with the development of the symptoms of pulmonary involvement in 1931 suggests the possibility that the condition was primary systemic moniliasis at the onset. Noteworthy was the remarkable improvement in general health as well as in the symptoms of pulmonary involvement after the administration of tincture of iodides. The case well represents one in which, through repeated attacks of fresh infection with the increasing extent of involvement, the malady may finally result in chronic pneumonia or the severe form (type II) of this disease, a possibility which probably was success-

fully prevented by the making of the correct diagnosis and by the specific therapy.

CASE 2.—A man aged 24, a worker in an iron foundry, was admitted to the hospital on Nov. 29, 1935, because of pain in the right side of the chest and elevation of the temperature. Five days before general malaise had developed. The patient suddenly became ill, with a temperature of 105 F., and had several chills. He was dyspneic.

Examination showed impaired resonance, with loud, rough, wheezing breath sounds over the right side of the chest. The temperature ranged from 102.4 to 105 F. The leukocyte count was 24,400. The sputum was blood-streaked and mucopurulent and showed many yeastlike organisms. Empyema developed in the right pleural cavity, with a secondary rise in temperature. The patient continued to cough and had occasional dyspnea. The empyema cavity was surgically drained on December 30. Thick, tenacious, greenish purulent fluid was obtained, which showed pneumococci in smears and M. albicans in cultures.

The history revealed that the patient had been told he had "hay fever" at the age of 4 years and had been treated for "bronchial asthma" during the past two years. He thought that the condition was aggravated by inhalation of dust in the foundry.

He was discharged from the hospital on Jan. 17, 1936. A month later he was still well. A small amount of mucoid sputum was obtained, from which M. albicans was isolated.

Comment.—The patient had been suffering from a mild form of bronchopulmonary moniliasis. Typical lobar pneumonia and later empyema developed. The isolation of M. albicans from the pus in the pleural cavity is of significance. There was a history of "bronchial asthma" for at least two years. The patient has remained free from asthmatic symptoms under iodide therapy since his recovery from pneumonia.

At this juncture, through the kindness of Dr. S. J. Lewis, of Beaumont, Texas, I obtained a large portion of the lung of a patient who had died of advanced bronchomoniliasis. Dr. Lewis [22] had published a report of this case in 1933. The study of this specimen prompted me to search in my own necropsy material for similar pulmonary lesions in which Monilia might be identified.

CASE 3.—A Chinese cook aged 50 was admitted to the hospital on Sept. 10, 1930, and died the following day of acute failure of the right side of the heart.

During the past three years the patient was admitted to the hospital on two occasions because of cardiac decompensation together with symptoms of advanced pulmonary disease, which was thought to be due either to tuberculosis or to a mycotic infection. The patient had been subject to "hay fever" for at least twenty years and had gone to Duluth, Minn., every August for relief. In 1928, while the patient was in Duluth, the abdomen began to distend, and the feet began to swell. The patient became short of breath, and he coughed considerably and perspired profusely. There was intense precordial pain.

22. Lewis, Seaborn J.: Am. J. Clin. Path. **3**:367, 1933.

When the patient was admitted to the hospital in September 1928 he appeared to be well developed and well nourished but extremely ill. His face and fingertips were cyanotic, and respiration was labored. The abdomen was tensely swollen, and the extremities were edematous. The patient preferred to sit upright in bed. The heart was enlarged to the left. No murmurs were heard. Loud, course and fine crackling râles and whistling and squeaky musical sounds were heard over the lungs. The liver was palpable and tender. There was pitting edema over the sacrum and the ankles. On Oct. 6, 1928, actinomycetes were said to have been found in the sputum, and the patient was given an intensive course of potassium iodide intravenously. An electrocardiogram showed weakness of the myocardium. A diagnosis of pulmonary tuberculosis or a mixed fungous infection of the lungs was made on the basis of roentgenograms. The patient was readmitted to the hospital in September 1929, with essentially the same complaints and findings. The sputum was fetid, copious, two-layered and typical of that obtained in cases of bronchiectasis or abscess. No tubercle bacilli were revealed on repeated examination. Several yellow cheesy granules in the sputum were found to be pseudosulfur granules consisting of a mass of bacteria.

Necropsy.—The heart weighed 430 Gm. The left ventricle was 1.5 cm. and the right ventricle 1 cm. thick. The wall of the right auricle also was thickened. Both chambers were dilated. There were no valvular lesions or coronary disease.

The right lung weighed 680 Gm. Crepitation was irregularly preserved throughout. The posterior portions were represented by areas of fibrosis, small cavities with brownish-green walls and evidence of pneumonic consolidation (fig. 1 *A*). The bronchi were dilated, and the walls were thickened and prominent. Thick, grayish, mucoid pus was expressed from the bronchi, cavities and consolidated areas. The main bronchus was filled with a tenacious, grayish, mucopurulent sputum. The left lung weighed 550 Gm. and showed an essentially identical picture, with extensive areas of emphysema along the periphery.

The following diagnosis was made: hypertrophy and dilatation of the right auricle and ventricle; purulent bronchitis, bronchiectasis and chronic fibrous pneumonitis; chronic passive congestion of the spleen, liver and kidneys, etc.

Microscopically, the pulmonary lesions were judged to be due to purulent bronchitis, bronchiectasis and chronic interstitial pneumonia (fig. 1 *B*). A detailed description is omitted because it would be similar to that to be given in connection with case 4.

By the Gram-Weigert method of staining the sections of the paraffin blocks and of the preserved lungs made more than five years before revealed infestation of yeastlike bodies, singly and in grapelike clusters, in the pulmonary lesions, regional lymph nodes and spleen. The organism was identified morphologically as Monilia (fig. 1 *C* to *E*).

Case 4.—A woman aged 72 was admitted to the hospital on May 8, 1931. She had had dyspnea since November 1930 and complained of a productive cough, excessive perspiration, weakness and loss of weight. She was discharged on May 22 and died at home on September 4.

The patient had pneumonia in January 1929 and had never fully recovered. In November 1930 she had an acute attack of chills, fever, dyspnea and coughing and complained of a sense of fulness in the left side of the chest. During the past three months there had been a productive cough and excessive perspiration. The patient had become weaker and more dyspneic.

The patient appeared to be well developed and well nourished. She became dyspneic on slight exertion. The chest showed slight hyperresonance, and there

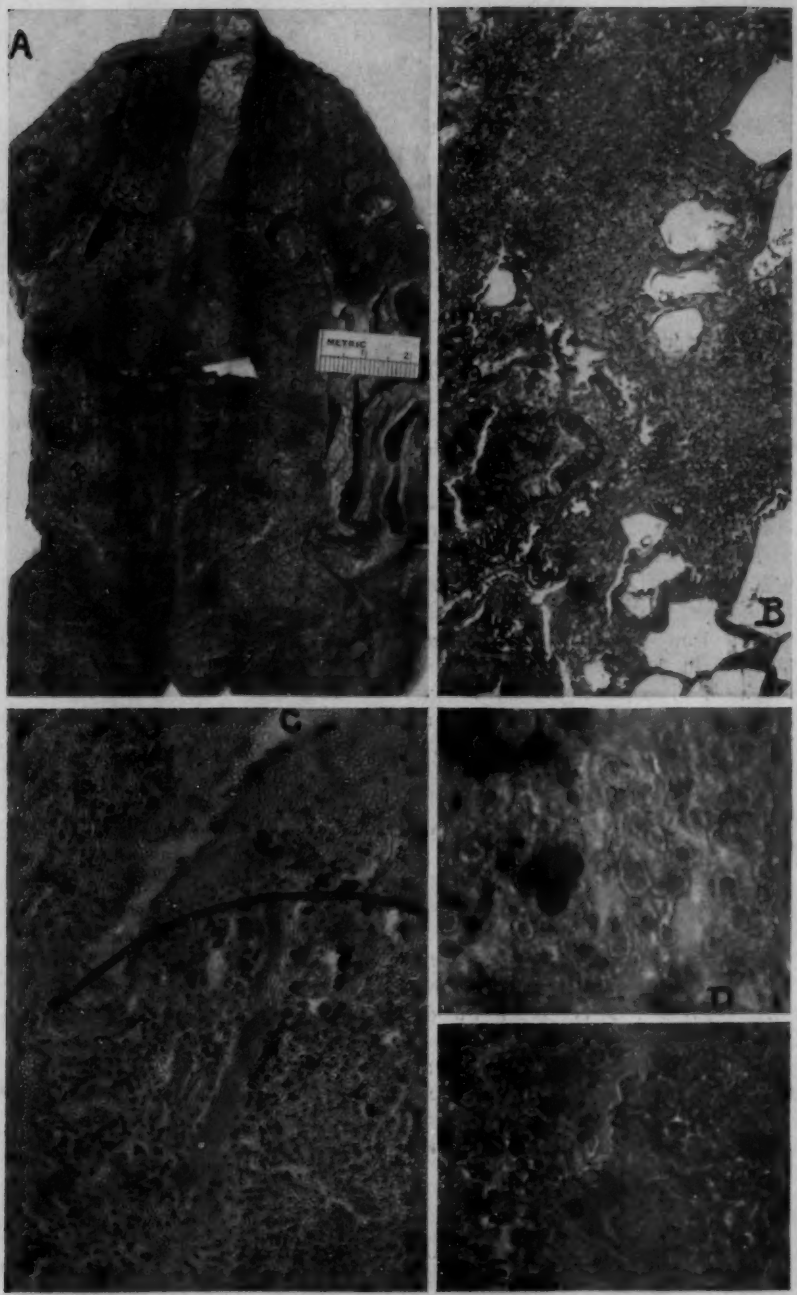

Fig. 1 (case 3).—*A*, sectioned surface of preserved lung, showing well defined cavities, bronchiectasis and areas of fibrosis, congestion and pneumonia. *B*, low power view of a more fibrous area, showing scattered lymphocytic infiltration, distorted and dilated alveoli, separated by thickened and fibrous septums, and a small bronchus presenting epithelial proliferation. Hematoxylin and eosin stain; × 40. *C*, a field from the lung, showing nests of yeastlike cells of Monilia. Modified Gram-Weigert stain; × 100. *D*, a small mass of monilia cells in a regional lymph node. Modified Gram-Weigert stain; × 430. *E*, a small clump of monilia cells in the spleen. The section was made from a preserved paraffin block prepared in 1930. Modified Gram-Weigert stain; × 430.

were fine râles over both lungs. The heart was essentially normal. There was definite clubbing of the fingers and toes. Examination of the nasal accessory sinuses revealed cloudiness of the left frontal and maxillary sinuses. Roentgenograms of the chest (fig. 2*A*) showed a soft, snowstorm-like mottling diffused throughout both lungs, with conglomerate shadows in the lower portions. The shadows were fairly uniformly distributed, with honeycomb areas of aeration intervening. There were no cavities. A blood count showed: hemoglobin, 75 per cent; white blood cells, from 10,550 to 12,300, and lymphocytes, from 62 to 69 per cent. The amount of sputum expectorated ranged from 3 to 15 cc. It was yellowish white, mucopurulent and odorless. No tubercle bacilli or fungi were seen in smears on repeated examination. The sputum became more purulent before death. No eosinophils were noted. The temperature ranged from 97 to 98.9 F. The pulse rate ranged from 70 to 88.

The patient returned home and was fairly well until three days prior to death, when she began to show signs of acute decompensation and anoxemia.

Necropsy.—The heart weighed 350 Gm. The right ventricle showed slight dilatation and hypertrophy, and the coronary arteries showed slight intimal atherosclerosis.

The right lung weighed 700 Gm.; the left lung, 550 Gm. They were diffusely nodular on palpation. Crepitation was greatly and equally reduced throughout. The parenchyma was rubbery and resilient in consistency. The cut surface revealed a somewhat uniformly dark red parenchyma, studded with grayish-white nodular areas, a few millimeters in diameter, slightly raised from the cut surface and usually surrounding a small bronchus. The surrounding parenchyma was diffusely fibrous, congested and partly collapsed. There were also areas showing small air-filled cavities, sometimes trabeculated. Several of these cavities showed a roughened inner lining and presented an appearance of bronchiectasis. Occasionally droplets of pus were obtained on pressure. Some of the larger bronchi showed thickening of their walls, with apparent inflammatory infiltration. The bronchial mucosa was swollen and congested (fig. 2*B*).

The following diagnosis was made: chronic interstitial pneumonia, with areas of bronchiectasis and emphysema, hypertrophy and dilatation of the right ventricle; coronary sclerosis, and chronic passive congestion of the liver, spleen and kidneys.

Numerous sections taken from different parts of the lungs were studied microscopically, and they presented grossly various stages of the disease. In several of the sections the lesion indicated the presence of an active process, with peribronchial and peribronchiolar zones of congestion and infiltration with many inflammatory cells. There were numerous dilated capillaries and diffuse invasion of plasma cells and proliferation of young fibroblasts. The bronchial and bronchiolar epithelium was swollen and showed active proliferation and desquamation into the lumen. Many of the lumens were irregularly dilated and contained precipitated muco-albuminous coagulum with few cellular elements. Here and there metaplasia of the lining epithelium was seen. Only a few alveoli were recognizable in the affected areas, the majority being collapsed or replaced by the increased connective tissue elements and infiltrates, together with edema and congestion. The alveolar septums were thickened by the increase in the connective tissue fibers. The septal cells were swollen, some being desquamated within the alveoli. Some areas were almost completely transformed into masses of connective tissue with little cellular infiltration. The blood vessels showed changes indicative of chronic inflammation. Elastic fibers were increased in number and clumped into masses. No micro-organisms were demonstrated (figs. 2*C* and 3*A*).

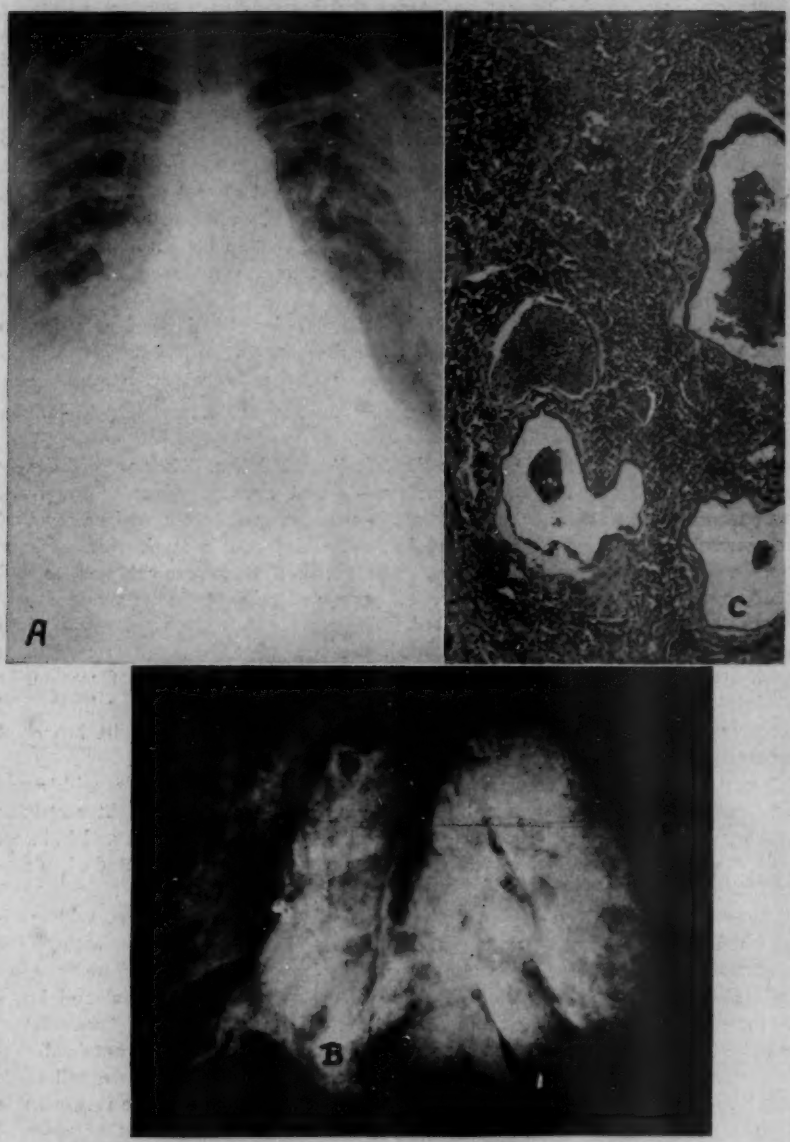

Fig. 2 (case 4).—*A*, roentgenogram of the chest taken on May 8, 1931. Diffuse, soft, mottled shadows are scattered all over both lungs, with conglomeration of the shadows in the bases. *B*, roentgenogram of the removed lungs. Marked fibrosis is indicated by the resistance to the roentgen rays. There are areas of emphysema and small cavities. *C*, an area of chronic pneumonia and bronchiectasis. There is infiltration of plasma cells. Note an albuminous type of exudate within the cavities. Original Gram-Weigert stain; × 40.

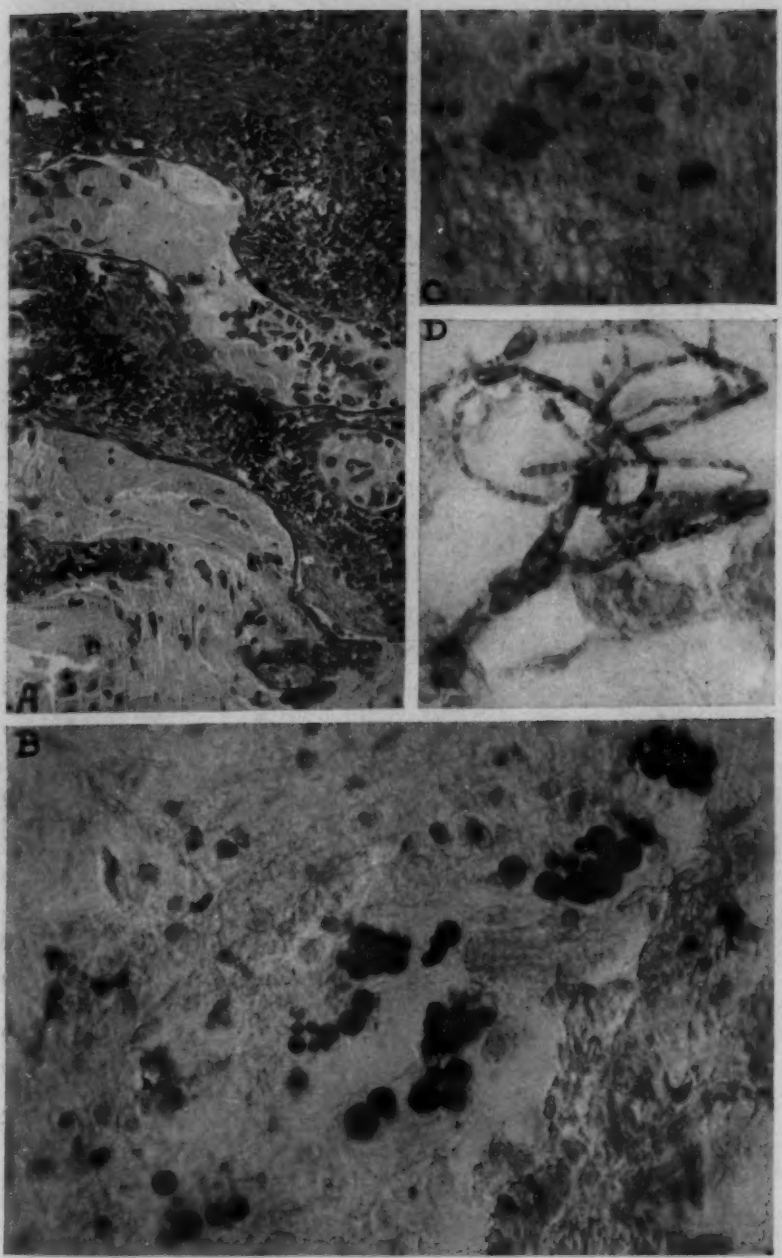

Fig. 3 (case 4).—*A*, rich plasma cell infiltration in a fibrous area. Dilated and distorted small bronchi or bronchioles are filled with a mucinous material and a number of macrophages. Original Gram-Weigert stain; × 100. *B*, clusters of monilia cells in the border of a pneumonic area. Modified Gram-Weigert stain; × 430. *C*, a cluster of monilia cells in a regional lymph node. Modified Gram-Weigert stain; × 430. *D*, mycelium showing chlamydospores in the capsule of a lymph node. Modified Gram-Weigert stain; × 430.

Sections of the old blocks and the preserved specimens stained with the Gram-Weigert stain revealed the presence of monilial bodies clustered in the pulmonary lesions and in the regional lymph nodes. A mass of mycelial filaments showing chlamydospores were seen along the capsule of one of the regional lymph nodes (fig. 3 *B* to *D*).

Comment.—M. albicans was not identified in the sputum during life in cases 3 and 4, no special cultural attempts having been made to isolate the organism. However, when the case was viewed in the light of the mycologic and pathologic observations of the lung in the proved case of bronchomoniliasis, the demonstration of yeastlike fungi with the morphologic characteristics of Monilia in the otherwise typical pulmonary lesions and in the regional lymph nodes (and in the spleen, in one case) seemed reasonably sufficient to establish these cases as examples of bronchomoniliasis.

Emphasis should be laid on the fact that it is practically impossible to demonstrate the organism in sections stained with hematoxylin and eosin. This accounts for the failure to recognize the organism during a routine examination of tissue. Monilia was demonstrated in the pulmonary lesions in twelve of forty control cases in which death was due to advanced tuberculosis with cavities, cancer [3] and other infections.[6]

Case 5.—A woman aged 47 was admitted to the hospital on Jan. 26, 1933, because of "chronic bronchial asthma." She died two days later, immediately after the bronchoscopic injection of iodized poppy-seed oil 40 per cent.

The patient had suffered from chronic asthma for more than fifteen years. She was first admitted to the hospital in August 1927, for the removal of polyps from the maxillary antrum. Polyps were again removed several times during the summer of 1932. The histologic examination of these polyps showed a highly edematous mucosa freely infiltrated with eosinophils (fig. 4 *A*). The usual remedial measures had failed to relieve the asthmatic condition.

Bronchoscopic examination showed the mucosa of the trachea and bronchi to be swollen. The lumens were filled with a thick, gray, gelatinous mucus. This was aspirated, and 20 cc. of iodized poppy-seed oil was instilled into the lower bronchus of the right lung. Death occurred within ten minutes.

Necropsy.—The heart weighed 300 Gm. and showed slight hypertrophy of the right ventricle, with relative dilatation of the chamber. The coronary arteries showed no evidence of disease.

The lungs were emphysematous throughout. Crepitation was well preserved. The pleural surfaces were smooth and pinkish. The cut surface revealed a soft, velvet-like parenchyma and prominent bronchi with markedly thickened walls, which stood out above the level of the cut surface. Nearly all these bronchi were solidly occluded by a grayish, tapioca-like, mucinous material. There were no areas of consolidation, and no pus was demonstrated. The mucosa of the main bronchus was swollen, injected and covered with patches of tenacious mucus.

The following diagnosis was made: chronic bronchial asthma, asphyxia, chronic bronchitis, emphysema and slight hypertrophy and dilatation of the right ventricle.

Microscopically, the bronchi showed marked proliferation and piling up of the epithelial lining cells, which were all goblet cells. There was a massive excretion of

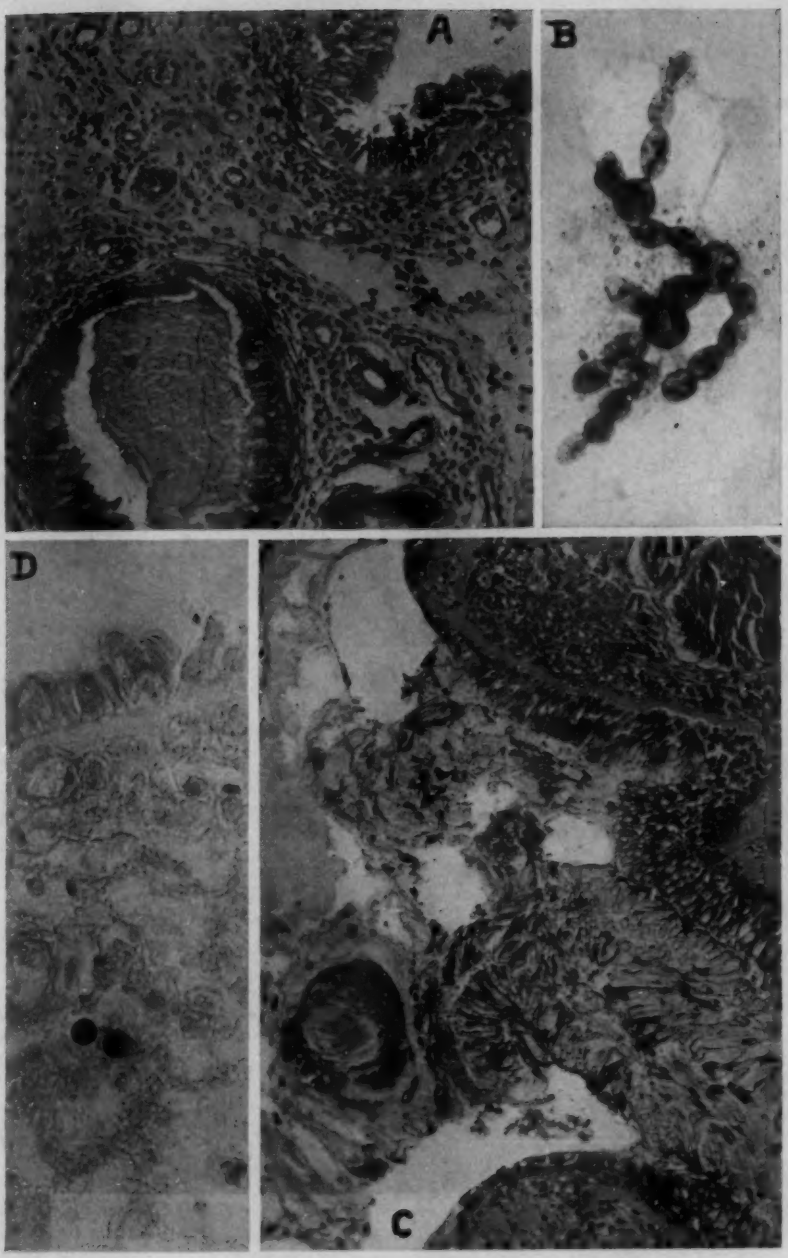

Fig. 4 (case 5).—*A*, section of the hypertrophic mucosa of the antrum, surgically removed, showing evidence of a typical allergic reaction. Hematoxylin and eosin stain; × 100. *B*, mycelium of Monilia demonstrated in the same section, with many yeastlike cells scattered throughout. Modified Gram-Weigert stain; × 430. *C*, section showing a typical field in an asthmatic bronchus. Hematoxylin and eosin stain; × 100. *D*, two monilia cells in the wall of a large bronchus. Modified Gram-Weigert stain; × 430.

mucinous material into the lumen. The mucin was loaded with eosinophils and a few exfoliated epithelial cells. Bandlike thickening of the bronchial basement membrane, hypertrophy of the muscle, diffuse infiltration of many eosinophils and few plasma cells were noted—a picture characteristic of bronchial asthma (fig. 4 *C*).

Sections of paraffin blocks of the necropsy material stained by the Gram-Weigert method revealed yeastlike organisms in the mucosa of the main bronchus and in the tracheobronchial lymph nodes but not in the pulmonary parenchyma. In the polypoid mucosa from the antrum, surgically removed in 1932, were also demonstrated numerous monilial bodies and mycelia (fig. 4 *B* and *D*).

Comment.—This case was included in this study largely because of the frequent reference made of the relation of bronchial asthma to monilial infection of the respiratory tract (Barnard [17] and Steinfield [23]). It is reasonable to assume that the condition represented an initial stage of bronchopulmonary moniliasis.

PATHOLOGIC ANATOMY

No lesions which may be construed as specific or peculiar to bronchopulmonary moniliasis have been described. However, there appears to be essential unanimity of opinion among observers regarding the cardinal changes which are present in the lungs and which may, at least partially, explain the pathogenesis of this condition. My own study of autopsy material substantially corroborates the observations of Boggs and Pincoffs,[2] Mendelson,[24] Joekes and Simpson [8] and Lewis.[22]

Mendelson,[24] who examined the lungs of a number of persons who suffered from this condition but who died of other causes, described the pulmonary lesions, which are somewhat comparable to those produced experimentally in animals. He recorded "the small tubercles" in the lungs, which "in reality, are mycotic tumors which stand out as very prominent white masses," and which, as a rule, show no signs of breaking down. This observation is of utmost importance, since there are no other records in which the early or nonfatal pulmonary lesions of moniliasis in man are presented.

At necropsy the lungs may show areas of partial collapse and emphysema. The pleura may be measurably thickened and adherent. The involved portions feel rubbery and lumpy on palpation. There are areas of pneumonic or nodular consolidation and fibrosis in which may be noted numerous small cavities and abscesses, presenting a honeycomb or cheeselike appearance. Larger cavities also may be encountered. The cavities and abscesses are usually in direct communication with the bronchi and represent dilated bronchi, bronchiectatic cavities or true focal necroses. The principal lesions are usually confined to

23. Steinfield, E.: J. Lab. & Clin. Med. **8**:744, 1923; J. A. M. A. **82**:83, 1924.
24. Mendelson, R. W.: J. A. M. A. **77**:110, 1921.

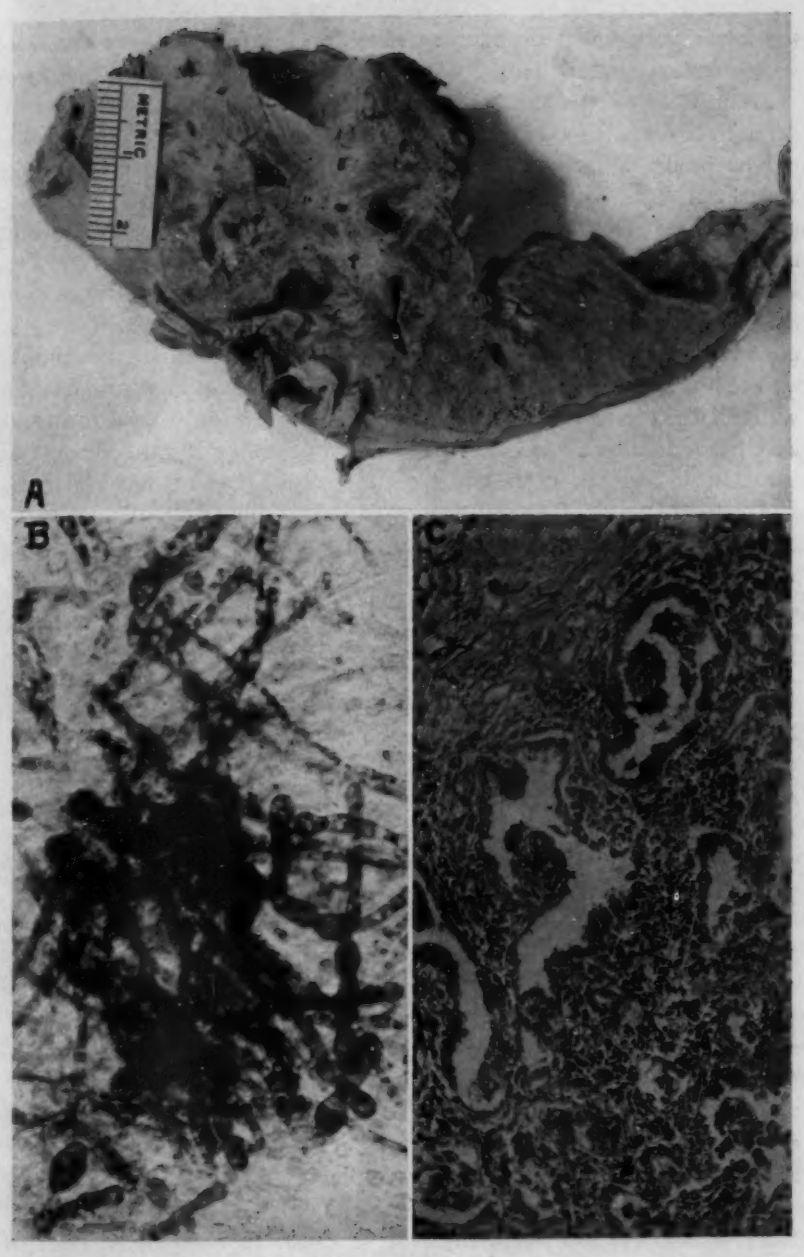

Fig. 5 (Lewis' case).—*A*, section of a portion of the preserved lung showing diffuse fibrosis, several abscesses and bronchiectasis and a greatly thickened pleura. This represents an advanced stage. *B*, large mycelium in the wall of an abscess in the same lung. Modified Gram-Weigert stain; × 430. *C*, marked proliferative reaction of the epithelium in a more recently involved area, with early interstitial fibrosis, edema and infiltration. Note the clumps of dark-staining monilia cells. Heidenhain's iron hematoxylin and tri-nitrophenol-fuchsin counterstain; × 100.

the lower portions of the lungs. The bronchi are filled with tenacious mucopurulent sputum (fig. 5*A*).

Microscopically, there is intense inflammation of the bronchi, both proliferative and suppurative, showing in some cases active proliferation of the lining epithelium and in others necrosis and ulceration of the superficial layer of the wall. The lumen may contain an acellular mucinous or albuminous coagulum or an exudate rich in plasma cells and macrophages. Eosinophils predominate in some cases, while a true purulent exudate is sometimes encountered. Complete obliteration of the alveoli and lumens of the smaller bronchi by coagulum may be observed. There is usually a heavy zone of peribronchial fibrosis and an accumulation of plasma cells. The necrotizing inflammation of the walls of the bronchi and bronchiectasis apparently lead to the formation of chronic localized abscesses. These are probably formed also by the invasion of Monilia in the areas of unresolved pneumonia. The yeast-like cells, often in budding forms and occasionally in filaments, are demonstrated in the cellular exudate in the wall of the abscess or of the inflamed bronchi (fig. 5*B*). They may be observed also in the less involved areas and in the regional bronchial lymph nodes. In the lymph nodes no local inflammatory reaction is noted.

The alveoli are distorted; many of them are dilated or contain a thick coagulum or a cellular exudate; others are collapsed or obliterated by edema, exudation and fibrosis of the septal stroma, which may be transformed into a widespread area of fibrosis with foci of cellular infiltration of varying intensity and extent. In these areas may be seen also numerous air spaces representing isolated alveoli. The alveolar epithelium may show active proliferation, with areas of localized metaplasia (fig. 5*C*).

The character of the cellular exudate may vary according to the type of the participating organisms. Without the secondary infection the chronicity and the low grade type of the inflammation favor the production of the plasma cells and mononuclear leukocytes. The macrophages are conspicuous within the alveoli of the bordering areas. Many of them are laden with fat droplets. Foreign body giant cells are occasionally encountered, and in some cases there are eosinophils. Where there are cavities and large abscesses with secondary invaders, the polymorphonuclear neutrophils may predominate.

Though perhaps secondary in importance, the changes in and about the vessels in the involved areas deserve special mention. Small arteries may show not only diffuse edema and thickening of the wall but also a heavy perivascular zone of round cell infiltration and fibrosis, presenting an appearance of miliary nodular periarteritis. Many of the larger arteries, on the other hand, may exhibit well organized subintimal swell-

ing and connective tissue proliferation, often to a point of obliteration, producing a high degree of chronic endarteritis.

ANIMAL EXPERIMENTATION

The yeastlike organism isolated from the sputum in case 1 of my series was reported by Dr. Stovall as giving "all the reactions which are recognized as characterizing Monilia albicans type. It produced only a few mycelial colonies; it fermented dextrose and maltose, with the formation of acid and gas. No gas was formed on saccharose. Milk was coagulated in three days." Dr. Henrici reported as follows: "Gas was formed on dextrose only. Maltose gave a little gas after three days. Milk was not coagulated; in fact, it slowly turned alkaline. A very slight mycelium was obtained in a gelatin stab culture after two weeks, but rather abundant mycelium developed in sucrose broth in twenty-four hours. I think that one is safe in stating that this is M. albicans." Dr. Shaw merely reported "M. albicans Robin, 1853."

Numerous rabbits were subjected to intravenous, intratracheal and intrapulmonary injections of this organism and of an organism isolated from pus from the pleural cavity of the second patient. The number of organisms approximated the lethal dose calculated by Stovall.[10] The rabbits, as a rule, died with uremia in from two days to a week. The blood urea nitrogen content rose rapidly to 175 mg. per hundred cubic centimeters of blood in three or four days, regardless of the route of injection. Miliary cortical abscesses of the kidneys were the most conspicuous abnormality in these animals. The heart, liver, spleen and brain also showed miliary foci of necrosis, usually microscopic. Microscopically, these showed minute focal necroses or tubercles, many bordering on abscess formation, and revealed active mycelial growth. In the lungs the results were not constant. In many animals which received intravenous and intratracheal injections the lungs appeared normal grossly at the time of death a few days later or when the animal was killed many days later, but microscopically there were occasional remnants of dead or broken filaments or masses of the organism, apparently caught in the net of the pulmonary capillaries. Occasionally in the animals which lived for several weeks, small tubercle-like formations were noted which were made up entirely of a collection of macrophages. These probably represent healed foci. At other times, small firm white nodules were grossly present in the lungs, usually along the main bronchovascular trunks. These were the true abscesses, and the organism was demonstrated in them in small numbers. There was a high degree of fibroblastic reaction around these abscesses, with active proliferation of the bronchial epithelium into the areas thus involved. The adjacent parenchyma showed varying degrees of inflammatory changes,

including a proliferation of the epithelium and an outpouring into the alveoli of many macrophages.

If a suspension of the organism was introduced directly into the lungs through the thoracic wall, the trauma thus produced and the subsequent extravasation and edema apparently favored the growth of the organism within two days and caused the development of acute pneumonia, with the formation of vascular thrombi and widespread dissemination of the monilial filaments, which freely penetrated the walls of the vessels and multiplied rapidly within the lumen. This undoubtedly favored the formation of thrombi. Within five days irregular white nodules were deposited over the pleural surfaces. A few nodules extended into the substance of the lung. In two weeks the entire pleural surface was transformed into a thick bed of white cheesy material, owing to the coalescence of the nodules. Monilial filaments in palisade formation sometimes bordered the cheesy mass.

The organism isolated in my cases was apparently of a highly virulent strain, and it induced extensive pleuropulmonary lesions in rabbits in a few days. With this strain of M. albicans, it is believed that the preliminary sensitization of rabbits, as employed by Kurotchkin and Lim,[25] may not be necessary in order to obtain the advanced pulmonary lesions with cavities which they were able to produce in their sensitized animals.

COMMENT

In the literature bronchopulmonary moniliasis has been accepted as an entity. In the early stage of the disease the organism apparently maintains a more or less saprophytic existence in an area of low grade inflammation, perhaps contributing by its presence toward the persistence or recurrence of the process. As a clinical syndrome, the condition when fully established appears to fulfil all the requirements. A few cardinal features may be recounted to justify this view: (*a*) a history of recurrent respiratory symptoms which simulate those of chronic bronchial asthma or advanced pulmonary tuberculosis; (*b*) the tenacious, mucopurulent sputum; (*c*) the persistent absence from the sputum of the tubercle bacilli and the presence, usually in large numbers, of a pathogenic species of Monilia; (*d*) the extensive nontuberculous chronic pulmonary lesions revealed in roentgenograms of the chest in the well established cases; (*e*) iodides, almost as a specific for the condition, and (*f*) the reproduction of identical pulmonary lesions in laboratory animals. Structurally, there are the exudative and necrotizing bronchitis and bronchiectasis; the nonspecific granulomatous inflammation leading

25. Kurotchkin, T. J., and Lim, C. E.: Proc. Soc. Exper. Biol. & Med. **31**: 332, 1933.

to chronic interstitial pneumonitis and multiple abscess and the constant presence of the yeastlike fungi in these lesions and in the regional lymph nodes.

On the other hand, because of the saprophytic nature of this organism, which is universally found in man, many prefer to believe that it is probably a secondary invader in the lesion and that it may merely contribute to the continued chronicity of the lesions.

It seems reasonable to postulate, however, that, though this fungus is normally relatively nonvirulent for man, given a lesion and conditions (perhaps chemical) favorable to its unrestrained growth, it may assume the rôle of an etiologic agent which is distinctly pathogenic in man, with potentialities of systemic dissemination. Facultatively pathogenic, the organism may thus cause local invasion and destruction; it may penetrate the walls of the bronchi and blood vessels and enter the areas of pneumonia, preventing resolution and producing foci of necrosis and abscesses until chronic pneumonia of an unusually complex type is established. This condition is designated as bronchopulmonary moniliasis. Kotkis and his co-workers [26] expressed a view which is in line with this conception. However, they gave the added opinion that there exists "some other possible factor in the causation of the chronic inflammation." One such factor may be found in the experiment of Kurotchkin and Lim,[25] who suggested individual or local tissue sensitivity as a cause of susceptibility.

SUMMARY

Since Castellani's observation in 1905, bronchopulmonary moniliasis has been considered as a clinical entity. From a purely clinical point of view, it may conveniently be so regarded.

The present study points to the existence of an apparent relationship between a pathogenic species of Monilia and an advanced fibrosing pneumonitis (chronic interstitial pneumonia) of obscure etiology, associated with bronchiectasis and abscesses.

The study also suggests a sequential relation of a certain type of chronic bronchial asthma to bronchopulmonary moniliasis; pathologically, such bronchial asthma may be regarded as an early phase of bronchopulmonary moniliasis.

It is, therefore, concluded that a pathogenic species of Monilia may be an important etiologic factor in the causation of chronic pulmonary infection of otherwise obscure origin; this is recognized as bronchopulmonary moniliasis; but it cannot be definitely stated that the organism is the sole cause of this condition.

26. Kotkis, A. J.; Wachowiak, M., and Fleisher, M. S.: Arch. Int. Med. **38**:217, 1926.

EXPERIMENTAL SIDEROSIS

III. SPECTROSCOPIC STUDIES OF IRON-CONTAINING PIGMENT

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BOSTON

Previous studies by one of us have demonstrated that an iron-containing pigment, indistinguishable from hemosiderin by known criteria, is readily produced in the spleen, bone marrow and liver after repeated intravenous injections of diluted ferric chloride.[1] Furthermore, the production of this pigment by the addition of the iron salt to tissue culture of the lymph nodes rules out its origin as a product of the degradation of hemoglobin.[1b] The view was expressed that the formation of iron-containing pigment is not referable solely to the breakdown of hemoglobin but may be the direct result of the intracellular digestion of phagocytosed iron-containing material present in the medium surrounding mononuclear phagocytes. For this reason cytosiderin was proposed as a more inclusive and far more accurate designation for iron-containing pigments than hemosiderin.

The literature contains reports of observations to the effect that hemosiderin may exist without any evidence of degradation of hemoglobin. Sprunt pointed out that in hemochromatosis, a disease characterized by abundant deposition of hemosiderin, there is no evidence of destruction of red blood cells. He assumed that the pigment is formed independently of hemoglobin from the iron of cells during autolysis.[2] More recently Whipple [3] suggested that hemosiderin may be the result of a change in the fundamental pigment metabolism in the organism rather than a product of the partial degradation of hemoglobin. It is even conceivable that the abundance of iron pigment in the liver in pernicious anemia may likewise prove to be referable

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1. (*a*) Menkin, V.: Proc. Soc. Exper. Biol. & Med. **31**:755, 1934; (*b*) Arch. Path. **19**:53, 1935. (*c*) Menkin, V., and Talmadge, S. M.: ibid. **19**:61, 1935.

2. Sprunt, T. P.: Arch. Int. Med. **8**:75, 1911.

3. Whipple, G. H.: Arch. Int. Med. **29**:711, 1922.

to some such basic metabolic disturbance, especially in view of the relatively small amounts of cytosiderin deposited in other organs.

The iron-containing pigment cytosiderin, the formation of which may be induced experimentally by injections of ferric chloride, reacts as true hemosiderin in that it fails to be dissolved either by hydrogen dioxide or by potassium hydrate.[1a] Are the various iron-containing pigments encountered in a number of pathologic conditions one and the same substance resulting from the intracellular digestion of phagocytosed iron material? The answer to this question will eventually be supplied by means of microchemical analysis of the pigment. However, as mentioned in an earlier study,[1b] spectroscopic analysis of these pigments may reveal whether they differ from one another in ways which are indiscernible by the usual histopathologic procedures. The importance and possible applications of such studies are obvious. The object of this brief communication is to report comparative spectrographic observations on pigment induced by the administration of ferric chloride to rabbits and monkeys and a similar pigment obtained post mortem from tissues of human beings with hemochromatosis, pernicious anemia and chronic passive congestion of the lung. The results indicate that in all these cases the pigments show similar absorption spectra, which differ greatly from those of a solution of ferric chloride and those of the pigment as found in splenic tissue from a person with malaria.

The spectrographic technic was as follows:

Since it is not possible to extract the pigments and measure their extinction by one of the routine methods, a microspectrographic method was adopted. An image of the cells to be investigated spectrographically was thrown on the slit of a Bausch and Lomb constant deviation spectrometer with a camera attachment, and the spectra of these cells were photographed. As the source of light, an automatic arc lamp was used, and the entire system was alined so that the optical axes of the illuminating system, microscope and collimator of the spectroscope coincided. The slit of the spectrograph was approximately 0.1 mm. wide. As reference spectrum, that of a quartz mercury arc was used. The spectra were taken on Wratten and Wainwright metallographic plates, and the time of exposure varied from fifteen seconds to four minutes. The developing procedure was carried out under standard conditions.

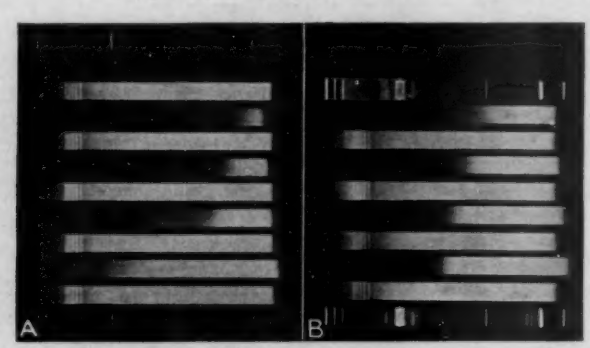
To prevent disturbing absorption from embedded material, the microscopic slides were prepared in the following way:

Nonstained microscopic sections of spleen from animals which had been given repeated intravenous injections of a 0.25 per cent solution of ferric chloride were mounted on slides. After the paraffin had been dissolved with xylene, a drop of paraffin oil was added to the section, and a cover slip was then applied. The same technic was employed with tissue from the liver and lungs of patients with various pathologic conditions, as mentioned previously. These specimens contained considerable amounts of pigment. Pigment was obtained also from the omental and mesenteric lining of a monkey which had been given intraperitoneal injections at weekly intervals for several months of a 0.25 per cent (isotonic) solution of ferric chloride. The omental membrane containing the pigment was carefully

teased out on a slide. The edges of the cover slip were sealed with a mixture of melted paraffin and petrolatum. To study ferric chloride under similar conditions, a drop of solution was placed on a slide and spread out under a cover slip.

A number of factors inherent in the spectrographic method, particularly when one is dealing, as in the present case, with very broad yet indistinct bands, cause the results obtained to be merely qualitative and valuable to the extent to which they are in agreement with those obtained by the previously mentioned methods. These variable factors are: scattering of light, variation in the thickness of the specimen, variation in the concentration of the pigment and variation in the sensitivity of the photographic plates according to the dye used to sensitize them to the visible spectrum. The method, however, should be much more valuable in connection with pigments having absorption spectra characterized by relatively narrow bands.

To control to a certain extent the variability of the first three factors mentioned, the following method was adopted: Spectra of pigment-containing cells were intercalated with those of unpigmented cells. As the pigments are transparent and the cells presumably the same, it can be assumed that the scattering produced by the cells is approximately the same. To make allowance for the



A, absorption spectra obtained with iron-containing pigment from hepatic tissue of a patient with pernicious anemia. *B*, absorption spectra obtained with a 0.25 per cent solution of ferric chloride.

varying thickness and concentration of the pigment, spectra of the pigment were taken with the exposure time varying from fifteen seconds to four minutes, while for the spectra of normal cells a constant time of exposure of thirty seconds was used. As the pigments are yellow-brown, their absorption will be least in the yellow portion of the spectrum. Consequently that spectrum of pigment was chosen to compare with corresponding spectra of the other pigments which showed the same degree of density in the yellow part of the spectrum as that of the normal cell.

The following results were obtained: The absorption spectra of the pigments produced by injections of ferric chloride, the iron pigment in the liver of a patient with pernicious anemia and of a patient with hemochromatosis and the iron pigment in the "heart failure" cells of a patient with chronic passive congestion of the lungs were found to be practically identical. The absorption begins in the neighborhood of 5,200 angstroms and increases very gradually toward shorter wavelengths, the

short wave limit of the spectrograph lying in the neighborhood of 4,000 angstroms, owing to the long path of the light in the glass of the whole system. The absorption spectra of the pigment from the patient with malaria and of the solution of ferric chloride are different. Because of its dark brown color the malaria pigment shows absorption over the whole range of the spectrograph. The absorption spectrum of ferric chloride begins at 4,800 angstroms and rises steeply toward shorter wavelengths. The spectra obtained are not suitable for reproduction. However, to illustrate the method, the accompanying figure gives the spectra obtained with the pigment from the liver of a patient with pernicious anemia and with a solution of ferric chloride, respectively. The figure shows the difference between the spectrum of ferric chloride and that of the pigment of pernicious anemia. The gradually increasing absorption of the pigment suggests that here the iron is bound to some large organic molecule, such as protein, for example.

CONCLUSION

The foregoing results indicate the identity of cytosiderin induced by repeated intravenous injections of ferric chloride with the iron-containing pigment observed in the tissues in cases of hemochromatosis, pernicious anemia and chronic passive congestion of the lung. The similarity of these pigments is therefore not merely a morphologic fact, but in view of the present observations it exists also in regard to their absorption spectra. This fact furnishes additional evidence, in accord with other known criteria described in the preceding communications of this series, that the iron-containing pigment resulting from the administration of ferric chloride is identical with that found in various pathologic conditions. Furthermore, there is no evidence of destruction of erythrocytes in hemochromatosis. The present observations substantiate the concept that the pigment cytosiderin, found in abundance in this disease, may be referable to a derangement of iron metabolism rather than being necessarily derived from degradation of hemoglobin.

Mr. Hayes, of the Boston branch of the Bausch & Lomb Optical Company, lent us the constant deviation spectrometer used in this study.

CAISSON DISEASE

A HISTOLOGIC STUDY OF LATE LESIONS

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CHICAGO

Paraplegia is only one of the signs observed in men with caisson disease on their return to normal atmospheric pressure after working in compressed air. Among the many names used to designate this condition ("diver's palsy," "bends," "compressed air illness" and "caisson disease") the term "aeropathy," suggested by Erdman,[1] is most appropriate, for it signifies the underlying pathogenic factor of the various symptoms incurred in caisson work and in occupations associated with similar hazards. Triger,[2] the French engineer, who was the first to operate a caisson successfully, in 1839, was also the first to emphasize, in a communication to the French Academy of Sciences in 1845, the pains in the extremities experienced by his men on their return to normal atmospheric pressure after a stay in compressed air.

A caisson is a steel cylinder which is sunk into water or water-containing soil, the water being kept out by means of compressed air. The men in the caisson are thus able to work on river bottoms and in similar places unhindered by the constant inflow of water. The top of the caisson is provided with an air lock, a chamber fitted with air-tight doors and cocks, wherein the air can become compressed or decompressed as the men leave or enter.

Pleomorphic as the symptoms of caisson disease are (pain in the extremities, commonly known as the bends, vertigo and dyspnea), paraplegia is the most striking. Most of the contributions to the pathology of caisson disease in general and to that of the spinal cord involvement in particular have pertained to cases in which the disease was relatively acute. This contribution deals with the changes in the spinal cord of a man who had had the disease for twenty-five years.

From the Pathology Laboratories (Dr. R. H. Jaffé, director) of the Cook County Hospital and the Division of Neuropathology (Dr. G. B. Hassin) of the University of Illinois College of Medicine.

1. Erdman, Seward: J. A. M. A. **44**:1665, 1907; Am. J. M. Sc. **145**:520, 1913.
2. Triger, M.: Compt. rend. Acad. d. sc. **13**:884, 1841; **20**:445, 1845.

REPORT OF CASE

History.—J. H., a colored man aged 63 years, had been a patient in the Cook County Hospital on several occasions over a period of three years because of cardiac decompensation. In addition he had a very interesting neurologic history, as follows: He had been employed for several years as a worker in a caisson and had experienced several attacks of the bends. These were not complicated by any permanent effects. An attack in 1909 was followed by gradual weakness of the legs, which developed within five hours into complete paralysis of both extremities and was associated with retention of the urine. The patient was confined to a hospital for one month, during which time he gradually regained partial control of the urinary bladder and some power in the lower extremities. When he left the hospital he was able to walk with the aid of crutches. During the following year he became sexually impotent. The slight improvement did not progress, and during the ensuing twenty-five years until the time of death he was able to get about only with the use of a cane.

Physical Examination.—The patient was a well developed colored man, lying in bed but not acutely ill. The temperature was 98.2 F., the pulse rate 82 and the blood pressure 200 systolic and 100 diastolic. The essential physical findings were marked cardiac hyperthrophy and moderate cardiac decompensation.

Neurologic Examination.—The pupils were unequal but reacted well to light and in convergence and accommodation. The cranial nerves were normal as were the speech and mental condition. The power and sensibility in both upper extremities were normal. Both lower extremities were spastic and offered great resistance to passive movements. The patellar and achilles reflexes were markedly exaggerated bilaterally, and the Babinski, Rossolimo and Chaddock signs were easily elicited. The abdominal and cremasteric reflexes were absent; the senses of pain, temperature and touch in the lower extremities were unchanged, and there was some diminution in the position sense in the toes. There was some difficulty in urination.

Laboratory Findings.—Urinalysis, blood counts and blood chemical examinations revealed nothing pathologic. The Kahn test of the blood was negative. Spinal puncture yielded a clear fluid under normal pressure with a negative Pandy reaction and no cells. The Queckenstedt test showed absence of a block. The Wassermann test of the spinal fluid was negative.

Course.—The neurologic condition remained unchanged, and on the last admission to the hospital the patient had very marked cardiac decompensation. He did not respond to treatment and died on Jan. 14, 1935. The final diagnosis was: hypertensive heart disease with cardiac decompensation and spinal cord degeneration caused by caisson disease.

Necropsy (Dr. R. H. Jaffé).—The anatomic diagnosis was: Very marked eccentric hypertrophy of the heart with mural thrombi in the apex of the left ventricle; marked sclerosis of the basilar cerebral arteries and slight atheroma of the aorta and coronary arteries; some form of chronic combined degeneration of the cord; moderate atheroma of the pulmonary arteries with a mural thrombus in the main branch of the right lower lobe; focal bronchopneumonia of the right upper and lower pulmonary lobes; catarrhal gastritis; mucous colitis; arteriosclerotic pitting of the kidneys; cloudy swelling of the liver; pitting edema of the lower extremities; slight ascites; nodose goiter; argentaffinoma and fibroma of the ileum.

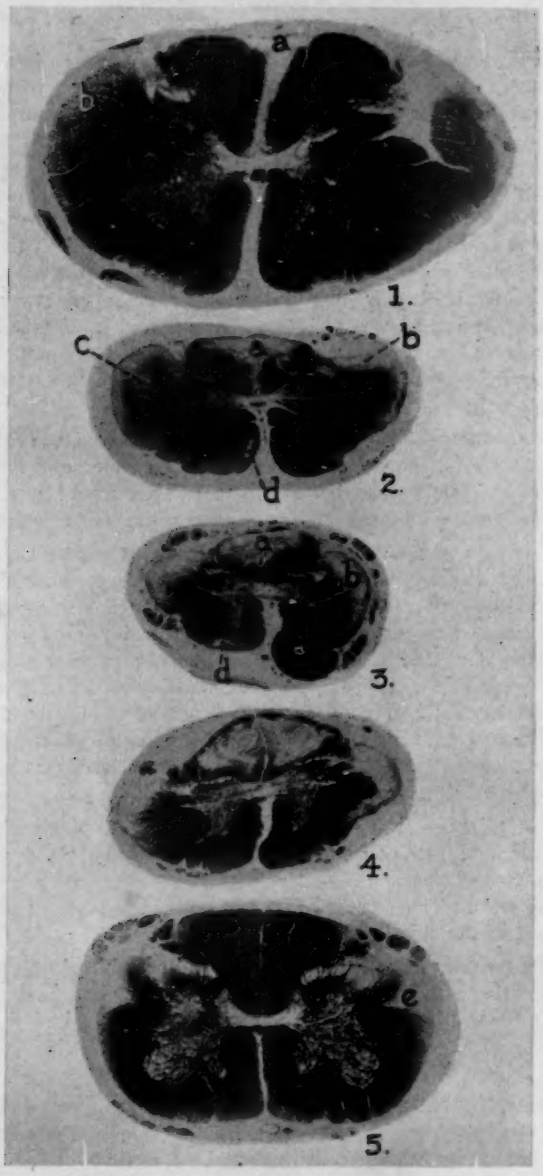

Fig. 1.—Involved segments of the spinal cord: In *1*, the fifth cervical segment, *a* indicates marked degeneration in the column of Goll and *b* milder degeneration in the dorsal spinocerebellar tract. In *2*, the second dorsal segment, *c* indicates diffuse degeneration in the lateral pyramidal tract and *d* a small area in the ipsilateral anterior column. In *3*, a middle thoracic segment, *a* indicates degeneration in the posterior column, *b* degeneration in the lateral column and *d* a small patch of it in the left anterior column. In *4*, a lower thoracic segment, more extensive degeneration is shown. In *5*, a lumbar segment, the changes were confined to both crossed pyramidal tracts (*e*).

Macroscopic Observations: The essential changes were in the central nervous system. The brain weighed 1,300 Gm., and in the basilar cerebral arteries there were numerous hyaline and calcified plaques. The spinal cord was of normal configuration, and the spinal canal presented no abnormalities. After fixation in a solution of formaldehyde the spinal cord was sectioned. The thoracic portion appeared much shrunken, and there were irregular glistening light gray-white patches in the posterior and lateral columns. In the cervical region similar changes were present in the column of Goll, and in the lumbar region, in the crossed pyramidal tracts. Sections were stained by the Weigert-Pal, Nissl, Alzheimer-

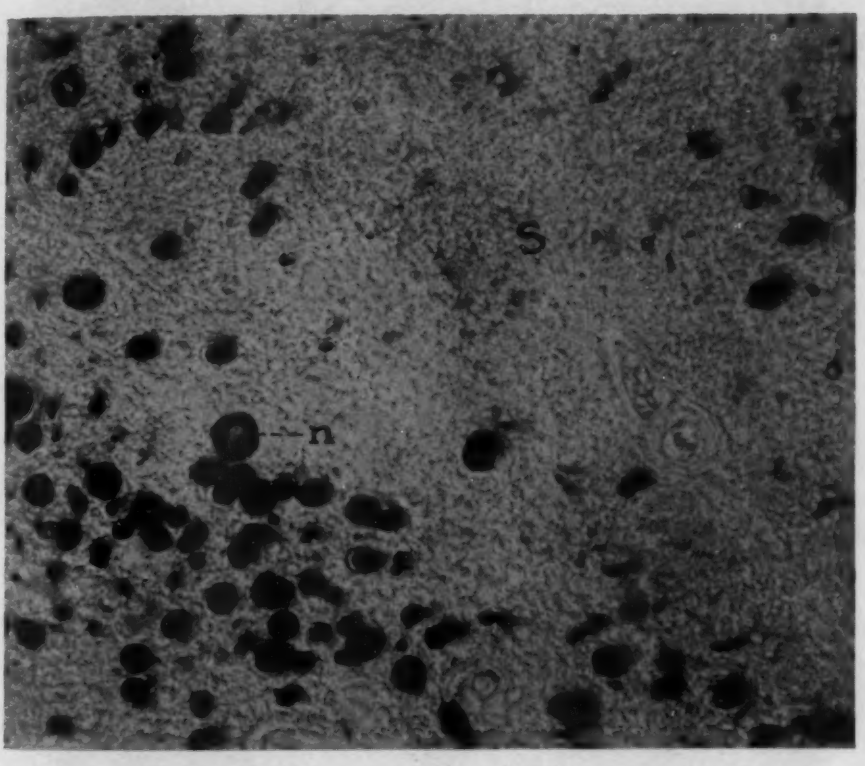

Fig. 2.—Transverse section through the posterior columns of the thoracic portion of the spinal cord. The glial scar *S* contains several nerve fibers, *n*. Weigert-Pal stain.

Mann, Mallory, Van Gieson, Bielschowsky and hemalum-eosin methods; frozen sections were also stained with sudan III.

Microscopic Observations: In sections stained with the Weigert-Pal method the white matter at all levels showed degenerative changes in the form of demyelinization of various tracts (fig. 2). The degeneration was most extensive in the middle and lower thoracic segments of the spinal cord, where it involved more or less the anterior, posterior and lateral columns (fig. 1*3*) but was by no means confined to the various systems of long nerve fibers.

For the most part the demyelinated fibers were located midway between the pia and the gray matter. At the level of the fifth cervical segment (fig. 1*1*) the column of Goll (*a*) appeared markedly degenerated, and only a small rim bordering on the pia remained intact. In addition there was somewhat milder degeneration of the dorsal spinocerebellar tract (*b*). In the second dorsal segment (fig. 1*2*) there was also diffuse degeneration of the lateral pyramidal tract (*c*) and of a small sharply circumscribed area in the ipsilateral anterior column (*d*). The middle thoracic portion (fig. 1*3*) exhibited degeneration of approximately two thirds of the posterior columns (*a*) with preservation of the ventrocom-

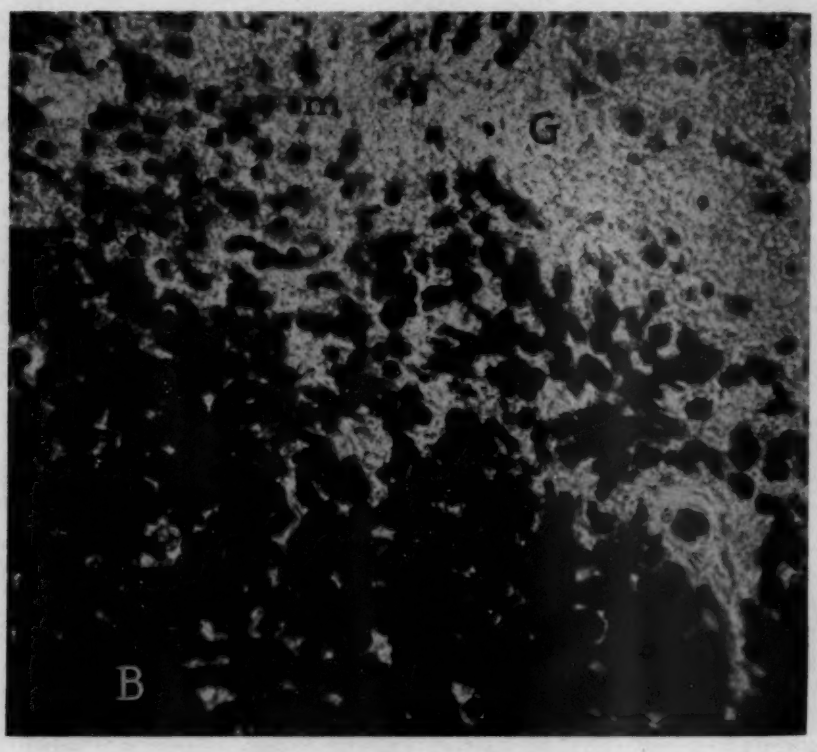

Fig. 3.—Transverse section through the posterior columns of the cervical portion of the spinal cord. Contrast the normally appearing Burdach's tract, *B*, with the degenerated Goll's tract, *G; m*, myelinated nerve fibers. Weigert-Pal stain.

missural zone and of a small marginal rim, of the lateral columns (*b*) and of a small patch in the left anterior column (*d*). In the lower thoracic portion (fig. 1*4*) degeneration of the dorsal and lateral columns was even more extensive than in the midthoracic area, while in the lumbar region (fig. 1*5*) the posterior and anterolateral columns were normal. Here the changes were confined to both crossed pyramidal tracts (*e*).

In short, in the cervical and upper thoracic regions the degeneration was ascending, confined to the long ascending tracts of nerve fibers; in the lumbar

region the degeneration was descending, confined to the descending crossed pyramidal tracts, while in the middle and lower thoracic regions the foregoing types of degeneration were combined. In such areas of combined degeneration it was possible to detect with a high power lens even in specimens stained with the Weigert-Pal method numerous normal myelin fibers (fig. 2). Figure 2 conveys at *s* an impression that such normal nerve fibers are scattered over smaller foci

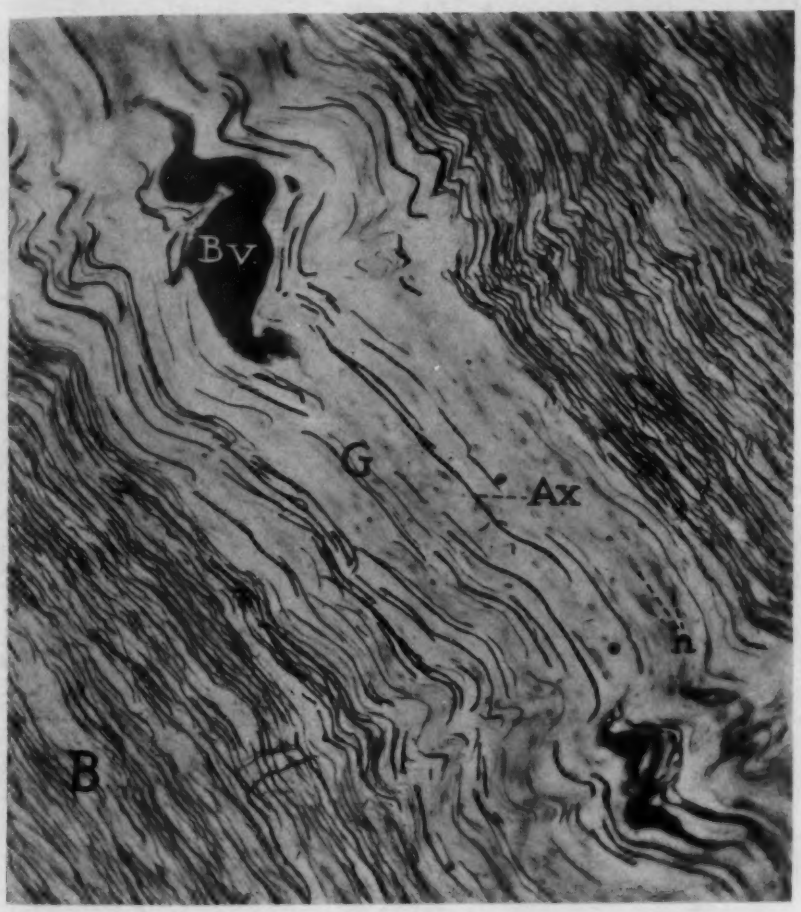

Fig. 4.—Longitudinal section of the cervical region of the spinal cord. *G* indicates Goll's tract; *B*, Burdach's tract; *n*, nuclei of fibrillary astrocytes; *Bv*, a blood vessel; *Ax*, an axon. Bielschowsky's stain.

which by coalescing grow into larger patches. Such miliary and even larger foci stained with the Bielschowsky, Alzheimer-Mann or Mallory method revealed also large numbers of fibrillary astrocytes, the processes of which intertwined, forming a dense glial network.

Though in segments of the cord stained by the Weigert-Pal method the glial scars were everywhere similar in appearance, in longitudinal sections stained with

the foregoing methods, especially that of Bielschowsky, there was at once apparent an appreciable difference between the glial scars of the cervical (fig. 4 *G*) or lumbar region and those of the thoracic region (fig. 5 *Sc*). For instance, in Goll's tract (fig. 4 *G*) the scar was in the form of longitudinal bands of sclerosis which followed the course of the nerve fibers. It was in the nature of the so-called isomorphous gliosis. The bands were separated from one another by preserved parallel running axons, and the nuclei of their fibrillary astrocytes (*n*) were also arranged in parallel rows. In contrast, as figure 5 shows, were the aspects in the thoracic region. Here, at *Sc*, the scar extending upward was like a dense

Fig. 5.—Longitudinal section of the thoracic region of the spinal cord. *Sc* indicates a solid glial scar; *S*, a glial scar similar to that shown in figure 4 at *G*; *Bv*, a blood vessel; *Ax*, an axon. Bielschowsky's stain.

feltwork and consisted of immense masses of thin fibrils transformed into a solid glial scar. Some fibrils in the scar exhibited a parallel course, but the bulk of the scar rather resembled a whorl formation. Glia nuclei were scarce here, irregularly scattered, and axons were altogether absent. The resulting gliosis may be termed anisomorphous in contrast to that seen in the scar *S* adjacent to it in figure 5. In the latter area the changes were similar to those in the columns of Goll described as isomorphous. The scars of anisomorphous gliosis took place

only in the thoracic region. There were present in the areas of scar formation numerous capillaries and blood vessels (*Bv*) of moderate size, their adventitial walls markedly thickened.

The various degenerated columns in the cervical region are well represented in figure 6. The columns of Goll (*1*) appeared much more severely involved than the dorsal spinocerebellar tract (*5*), while the pyramidal tract (*4*) and the posterior horn (*3*) were not affected. Intense and extensive as the degeneration of the

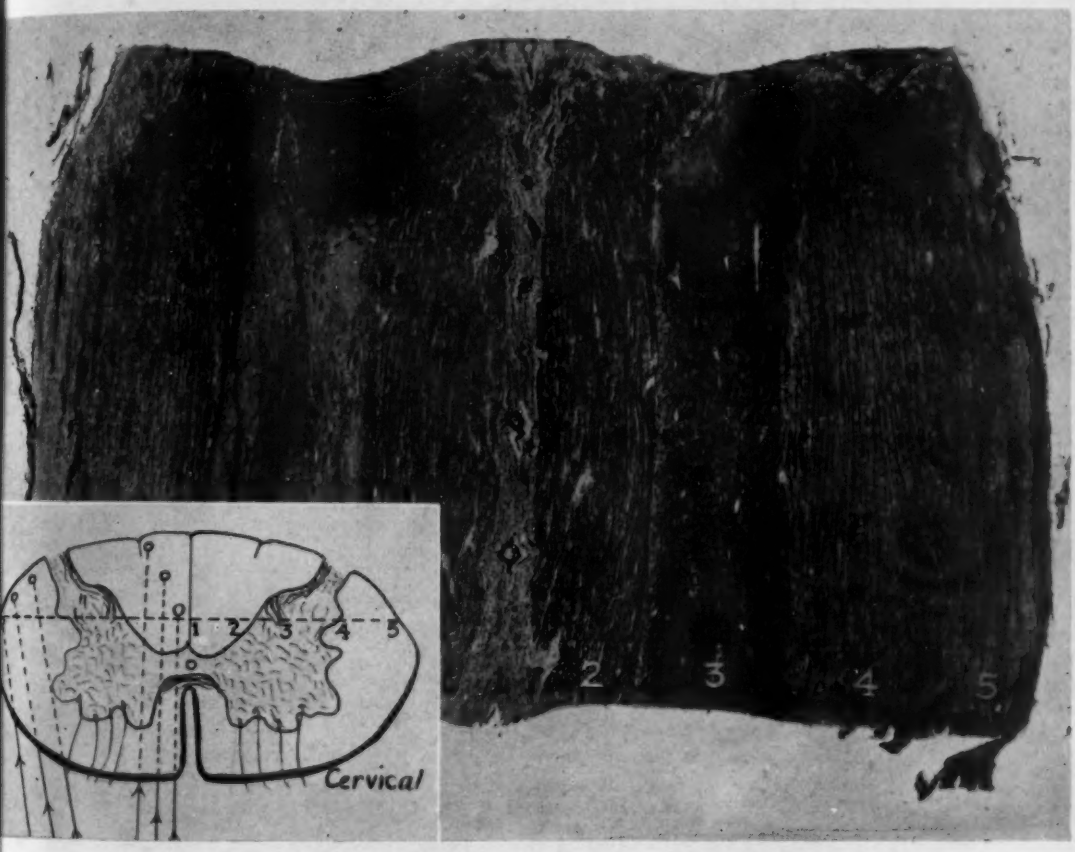

Fig. 6.—Longitudinal section through the posterior portion of the cervical spinal cord as indicated by the dotted line in the insert. The degenerated Goll's tract, *1*, and the partially degenerated dorsal spinocerebellar tract, *5*, stand out in contrast to the normally appearing Burdach's tract, *2*, the pyramidal tract, *4*, and the posterior horn, *3*. Bielschowsky's stain.

long fiber tracts was, it was secondary to a primary lesion in the thoracic region, which, as has been pointed out, was affected more diffusely than any other portion of the spinal cord. Thus the cervical and lumbar parts exhibited only secondary degeneration while in the thoracic region the degeneration was both secondary and primary. The latter type was represented by small foci of anisomorphous

gliosis scattered exclusively throughout the white columns of the thoracic segments of the spinal cord. In figure 1 (*2* and *3*) such scars appeared isolated in the anterior columns at *d* while in the posterior and lateral columns they were

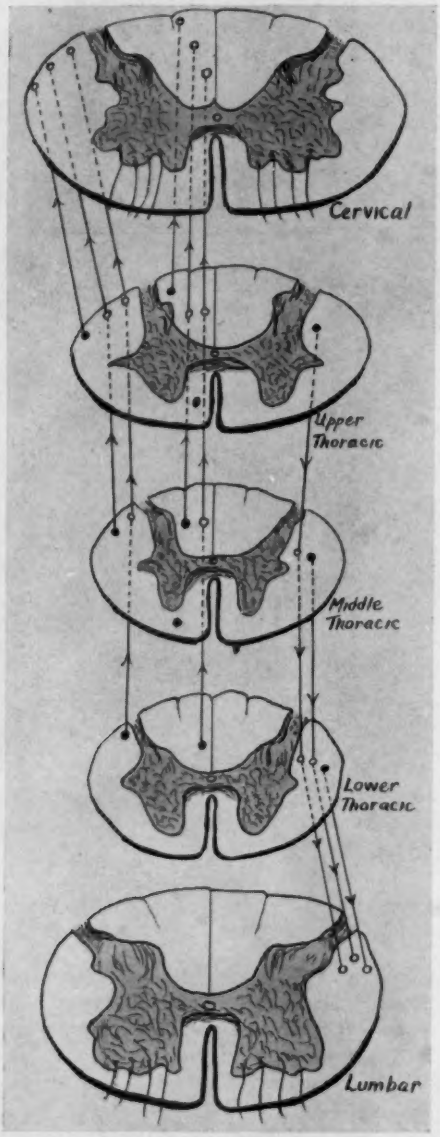

Fig. 7.—Diagram illustrating various levels of the spinal cord. Note the relationship of the scars of secondary degeneration, represented by the white circles, to those of primary degeneration, represented by the black circles, as indicated by the lines and arrows.

incorporated, as it were, within the scars of isomorphous gliosis which made up the secondary degeneration of the long fiber tracts. These scattered small foci of nerve degeneration are shown diagramatically in figure 7, where they can be contrasted with the areas representing secondary nerve degeneration. The diagram illustrates the topography of the primary and secondary scars (white circles representing secondary degeneration; black circles, the primary glial scars) and their coexistence in the thoracic portion. This relationship is also well represented in figure 5, in which the glia scar (*S*) from secondary nerve degeneration, containing healthy axons (*Ax*), is adjacent to a scar (*Sc*) from a primary focus already described in detail.

The pia was markedly thickened throughout the entire extent of the spinal cord, and nowhere did it show cellular infiltrations. The arachnoid membrane in its ventral and dorsal portions was for the most part unchanged, but at its lateral aspects where it accompanied the nerve roots into the epidural space it exhibited an abundance of so-called arachnoid cells. These often appeared in clusters or as whorls containing calcified bodies, while in other places they formed only villous prolongations.

The spinal nerve roots embedded in these masses of proliferated cells were unchanged except for a moderate increase in the number of the arachnoid perineurial and endoneurial cells. The anterior and posterior spinal arteries as well as their coronal branches were patent throughout.

Sections from various portions of the brain revealed no noteworthy histologic changes except slight thickening of the meninges and moderate chromatolysis in the ganglion cells.

COMMENT

The changes outlined can be summed up as ascending and descending degeneration of the spinal cord secondary to a severe destructive lesion of its thoracic portion. The cause of the central nerve lesions in caisson disease is usually considered to be embolism of minute blood vessels, which causes degeneration of all the constituent nerve elements. Bassoe [3] classified cases of caisson disease in its late manifestations into three groups: (1) cases in which the spinal symptoms predominate—so-called caisson myelitis, (2) cases in which there is permanent involvement of the joints and (3) cases in which there is permanent involvement of the ears.

The common occurrence of spastic paraplegia in caisson disease can be explained by the changes in the spinal cord described. They certainly were not inflammatory, in the nature of myelitis, but degenerative. The question arises: What causes the degeneration?

Pathogenesis of Caisson Disease.—That air embolism may be the cause of the changes in caisson disease is self evident, and the embolism theory is now generally accepted through the work of Bert,[4] Hoppe-Seyler, Hill and McLeod [5] and others. It has been shown that the fluids and tissues of a man working in compressed air become saturated

3. Bassoe, P.: Tr. Internat. Cong. Hyg. & Dermog. **3**:626, 1913.
4. Bert, Paul: La pression barometrique, Paris, Masson & Cie, 1878.
5. Hill, L., and McLeod, J. J. R.: J. Hyg. **3**:401, 1903.

with the gases of the atmosphere, depending on the length of exposure, the intensity of the pressure and the vascularity and absorptive ability of the individual tissues. Since 80 per cent of the atmosphere consists of nitrogen gas and since lipoids absorb about five times the amount of nitrogen the blood plasma is capable of carrying (Haldene, Vernon [6]) it is apparent that after prolonged exposure to compressed air the great lipoid reservoirs of the body (the accumulations of fat and the myelin of the central nervous system) become saturated with nitrogen gas. When the pressure is released the fluids and tissues give up their nitrogen into the circulating blood. The glands and muscles of the body, having an abundant vascular supply, give up this gas readily. When it is released in the arterial blood stream it rapidly passes through their capillary beds without producing infarction. Since the fat tissues and the white matter of the spinal cord have a meager blood supply as compared with their bulk they cannot rid themselves of the nitrogen gas as readily. The small bubbles of gas liberated in the blood stream enter the capillaries of the white matter. By absorbing nitrogen gas from the neighboring myelin laden with nitrogen the bubbles increase in size so rapidly that they obstruct the capillaries and their escape becomes impossible. Other bubbles of nitrogen arising from the myelin cannot enter the circulation because of the capillary obstruction, and thus tears in the nerve parenchyma occur.

In these respects animal experiments (Boycott, Damant and Haldane [7]) substantiated the phenomena observed in man. Histopathologic studies of the changes in the spinal cord in caisson disease indicate that the most vulnerable part is the thoracic region and that this is probably due to its peculiar blood supply. For this reason a short review of the blood supply of the central nervous system will illustrate why certain regions are especially sensitive to vascular disturbances.

Except in cases of very rapid decompression, the brain is immune to infarction because of its rich vascular supply. In the spinal cord the cervical enlargement is only rarely affected because of its ample blood supply through the anterior and posterior spinal arteries (the vertebral system). The lumbar enlargement is also rarely affected, as it is equally amply supplied by the arteria spinalis magna, which obtains its blood from the spinal branch of the hypogastric artery (the pelvic system). The extensive thoracic portion of the spinal cord, which is supplied by the small spinal branches of the intercostal and lumbar arteries (aortic system) and by anastomotic twigs from vessels to the cervical and lumbar enlargements, is readily seen to be in greatest jeopardy. The gray matter of the thoracic portion usually shows no changes both because of its rich vascular supply which prevents infarction and

6. Vernon, H. M.: Lancet **2**:691, 1907.
7. Boycott, A. E., Damant, G. C. C., and Haldane, J. S.: J. Hyg. **8**:342, 1908.

because of the small amount of myelin it contains and hence the small amount of nitrogen gas liberated. It is the central portions of the white matter, not the portion bordering on the pia, that show changes in moderate cases; the periphery is nearest and much better supplied by the penetrating branches of the vasa corona which nourish the white matter. The distribution of air bubbles in the spinal cord in experimental animals in which caisson paraplegia had been produced (Boycott and Damant [8]) can be explained by the vascular supply as presented. The changes in the spinal cord found in persons who died weeks and months after the onset of the condition have been shown by Leyden,[9] Erdman [1] and others to be congestion of the spinal cord, hemorrhages and "myelitis," all most marked in the thoracic portions.

As has been noted, the changes are not those of myelitis but those of obstruction of the blood vessels by gas emboli and result in softening. In caisson disease the emboli occlude vessels of capillary size and only minute areas of softening occur (myelomalacia). Such myelomalacia may be termed miliary, that is, the injury of the cord is so small that though permanent it is compatible with life.

The softened tissues of the cord are transported as lipoids along the Virchow-Robin perivascular spaces to the subarachnoid space as has clearly been shown elsewhere.[10] In this case the perivascular (adventitial) spaces of Virchow and Robin contained no lipoids, as they had been long since discharged, leaving the adventitia and pia permanently thickened as the consequence of reactive phenomena. When the defect remaining after the removal of the softened parenchyma is small and the glia in the vicinity efficient, that is, unaffected by the agent causing the softening (toxins, for instance), it is capable of proliferating and forming a glial scar. The primary scars in caisson disease must therefore be miliary but very numerous.

The differentiation of these patches from those of multiple sclerosis is worthy of consideration. In the latter condition, though normal nerve fibers may be seen coursing through the dense glial scars, they are usually not myelinated, for the causative factors produce demyelinization very early and do not spare the myelin on any fiber implicated in the patch affected. Then, again, the apparently normal portions of the spinal cord adjacent to the glial scars in multiple sclerosis are seen to be the site of preliminary degenerative changes such as tumefaction of the myelin and the presence of myeloclasts and myelophages (Hassin [11]). In caisson disease the tissue adjacent to the scars is entirely normal.

8. Boycott, A. E., and Damant, G. C. C.: J. Path. & Bact. **12**:507, 1907-1908.
9. Leyden, E.: Arch. f. Psychiat. **9**:316, 1879.
10. Zeitlin, H., and Lichtenstein, B. W.: Arch. Neurol. & Psychiat., to be published.
11. Hassin, G. B.: Arch. Neurol. & Psychiat. **7**:589, 1922.

SUMMARY

Chronic combined degeneration of the cord due to aeropathy (caisson disease) of over twenty-five years' duration is presented.

The thoracic oprtion of the spinal cord is the site of prevalent involvement, and of this the white matter is very much more affected than the gray.

The old scars resulting from the embolic occlusion of the capillary blood vessels are exclusively ectodermal and characterized pathologically as anisomorphous gliosis.

This condition is best differentiated histologically from multiple sclerosis by detailed study of the areas adjacent to the scars and of those of normal remote regions.

IMPORTANCE OF ALLERGY AND IMMUNITY DUE TO A GHON TUBERCLE IN PATHOGENESIS OF EXPERIMENTAL PULMONARY TUBERCULOSIS

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The significance of allergy and immunity resulting from a primary pulmonary tuberculous infection (Ghon tubercle) even though the primary infection has apparently healed has had various interpretations. Whether a primary healed pulmonary lesion with associated allergy should be considered a useful or a harmful factor is not agreed on at present by students of tuberculosis. Investigators who have written on this aspect of tuberculosis fall into two groups.

The first group has emphasized the harmful effects of allergy caused by the existence of the Ghon tubercle. The more severe secondary pulmonary infection or fatal tuberculosis is looked on as the result primarily of existing allergy due to the Ghon tubercle. Immunity, if present, is believed to be almost masked or overcome by the bad influence of allergy. As proof of these beliefs the fact is stated that practically all pulmonary tuberculosis severe enough to cause death is preceded by primary pulmonary infection.

Myers [1] may be cited as an advocate of the theory that the primary lesion is ineffective in helping to ward off further pulmonary tuberculosis. He stated:

> Our observations have convinced us that only those children with the first infection type of tuberculosis develop the adult and destructive type. Therefore, we cannot see our way clear to consider the first infection type of tuberculosis as a protection to the child.

Myers [2] emphasized the harmful aspect of allergy in the following statement:

> A positive tuberculin reaction, indicating allergy resulting from first-infection type of tuberculosis, is a definite liability, since the destructive forms of tuberculosis do not ordinarily develop in nature in its absence.

From the Department of Pathology, the University of Minnesota Medical School.

1. Myers, J. A.: Ann. Int. Med. **6**:672, 1932.
2. Myers, J. A.: Am. Rev. Tuberc. **28**:93, 1933.

Johnson and Myers [3] further stated:

> First-infection type of tuberculosis in infants, whether or not the lesion can be located, rarely if ever kills, but results in allergy which makes reinfection from exogenous or endogenous sources dangerous.

Stewart [4] was equally emphatic in stressing the harmful effects associated with primary pulmonary tuberculosis. He stated:

> The relationship existing between the childhood and adult type of tuberculosis seems to consist largely in the tendency for the primary infection to prepare the patient for the development of phthisis should he perchance later experience a reinfection of sufficient severity to produce an intrapulmonary lesion.
>
> The part that the former childhood infection seems to play in favoring the later development of phthisis seems to depend more upon the changes which the primary infection caused with respect to the manner in which the body reacts thereafter to the tubercle bacillus and its products, rather than upon a lighting-up of the old disease acquired during childhood.

Long [5] stated that the opinion is now growing more general that necrosis is largely the effect of the protein of the tubercle bacillus on a body made hypersensitive to the micro-organism by preexisting infection with the bacillus.

Rothschild, Friedenwald and Bernstein,[6] from experiments in desensitizing tuberculous guinea-pigs, concluded that allergy is responsible for necrosis and mechanical spread in tuberculosis.

The second group of investigators in tuberculosis is convinced that a degree of immunity (resistance) is associated with nonprogressive primary tuberculosis (Ghon tubercle). Allergy, while admitted to have a harmful aspect, such as responsibility for an increased degree of necrosis, should not be taken too seriously. In fact, it is sometimes stated that without allergy there is no resistance. To prove these assertions the fact is cited that in the greater number of persons having a Ghon tubercle clinical or anatomic tuberculosis does not develop again, even if the person is subjected to repeated infections.

Krause [7] stated:

> Immunity becomes a part of every tuberculous process as soon as the latter gets well under way. Indeed, . . . it directs, in a measure, most human infections received under the conditions erected by our civilization. . . . It acts as a force which is ordinarily sufficient to stay the unbridled effects of those numbers and types of bacilli that are the average for first infections of our civilization or fall below this average. . . . The allergy and immunity aroused by their [most civilized human beings] initial infections are competent to hold in abeyance and quiescence [the] focal tubercle.

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3. Johnson, W. M., and Myers, J. A.: Am. Rev. Tuberc. **28**:381, 1933.
4. Stewart, C. A.: Am. J. M. Sc. **185**:346, 1933.
5. Long, E. R.: Am. Rev. Tuberc. **22**:467, 1930.
6. Rothschild, H.; Friedenwald, J. S., and Bernstein, C.: Bull. Johns Hopkins Hosp. **54**:232, 1934.
7. Krause, A. K.: Am. Rev. Tuberc. **18**:208, 1928.

In respect to the pathogenesis of the adult type of pulmonary tuberculosis, Opie [8] stated:

> We may justly assume that the localization of the disease in the lung is referable to the immunity conferred by a first infection, and its tendency to pursue a chronic course with fibrosis may be similarly explained.

Petroff and Stewart [9] stated that it is now generally accepted that the resistance of animals to superinfection depends chiefly on the degree of the allergic state and that the absence of allergy, as demonstrated by the reaction to the intracutaneous tuberculin test, spells susceptibility to infection.

After an intravenous injection of a bovine strain of tubercle bacilli into normal rabbits and rabbits which had previously been infected with a human strain of bacilli, Lurie [10] found that from one to eight weeks later the number of bacilli which could be cultured from organs was decidedly less in the reinfected animals.

It has been difficult to harmonize the observations and conclusions of these two groups. It does not seem possible from observations on patients to evaluate the delicate balance between allergy and resistance in the pathogenesis of tuberculosis.

Four important factors should be considered: (1) the degree of allergy, (2) the virulence of the infecting organisms, (3) the frequency of reinfection and (4) the size of the reinfecting dose. The first factor can be determined in the patient, but there is no means of measuring the virulence of the organisms and the frequency and extent of reinfection. It cannot be believed that the virulence of the infecting bacilli can regularly be greater in reinfection than in primary infection. The two variable factors, then, which cannot be measured in human tuberculosis are the number of infections and the size of the infecting dose.

It seems that if a balance between allergy and resistance in the pathogenesis of tuberculosis is to be demonstrated, the explanation will have to come from experiments on animals, in which all the aforementioned possible variable factors can be carefully measured. It must be assumed that tuberculosis in animals and that in man progress for the most part in a similar manner. It is known, however, that tuberculosis in man tends to progress more slowly than tuberculosis in the rabbit. Rabbits were used in the experiments reported in this paper.

The experiments were undertaken to find a significant relation between allergy and resistance in the pathogenesis of pulmonary tuberculosis.

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8. Opie, E. L.: Am. Rev. Tuberc. **32**:617, 1935.
9. Petroff, S. A., and Stewart, F. W.: J. Immunol. **12**:97, 1927.
10. Lurie, M. B.: J. Exper. Med. **57**:181, 1933.

EXPERIMENTAL INVESTIGATION

Methods and Materials.—The culture of tubercle bacilli used in making the animals allergic was the BCG bovine strain. This strain was used because it was found that large quantities of the bacilli could be injected into rabbits without producing progressive tuberculosis; hence it was possible to reproduce closely in the rabbits the condition found in patients with healed or nonprogressive Ghon tubercles.

The animals were made allergic by the four following methods: (1) subcutaneous injection of 2 mg. of living BCG, (2) intravenous injection of a few (four or five) dried clumps of BCG; (3) intrapulmonary injection of 2 mg. of living BCG through a long needle in the trachea, and (4) intrapulmonary injections of 2 mg. of living BCG through the thoracic wall and pleura. For comparison with the results of these injections of BCG, another series of animals were given injections into the lung through the thoracic wall and pleura of 0.001 mg. of a virulent strain of bovine bacilli.

Allergy readily developed with all the methods in from two to three weeks. The degree of allergy was recorded in terms of +, ++, +++ and ++++, depending on the extent of the reaction, infiltration and necrosis, forty-eight hours after an intracutaneous injection of 1 mg. of old tuberculin.

Eight weeks after inoculation with BCG for the purpose of producing allergy, all the allergic animals and a series of normal animals were given subcutaneous injections of 0.01 mg. of a virulent strain (Ravenel) of bovine tubercle bacilli. All the animals were killed forty-five days after the injection of the virulent bacilli, and the degrees of tuberculosis in the lungs and kidneys of the normal and the allergic animals were compared. It had been observed in previous experiments that 0.01 mg. of the Ravenel strain injected subcutaneously regularly produced extensive tuberculosis in the lungs and often in the kidneys of the normal animals in forty-five days. The degrees of tuberculosis, if present, in the lungs and kidneys were expressed in grades of from + to ++++. Grade + represented from one to four tubercles, depending on the size. Grade ++++ indicated so many tubercles that they could not be counted. Grade ++ and +++ were intermediate between grade + and grade ++++.

Results of the Experiments.—1. Seventeen rabbits were made allergic by the first method (subcutaneous injection of living BCG). This group was included to determine whether results might be obtained which differed from those in the animals with allergy-producing lesions in the lungs. These seventeen animals and sixteen normal rabbits were then given injections of the virulent strain. At the end of forty-five days, when the animals were killed, all the normal animals showed tuberculosis in the lungs of grades ++ to ++++. Tuberculosis was present in the kidneys of all except three of the sixteen normal animals. The degree of tuberculosis in the kidneys of the thirteen animals having tuberculosis ranged from + to ++++.

In contrast to the observations on these normal animals, no tuberculosis was noted in the lungs or kidneys of any of the allergic animals. The degree of allergy in these animals ranged from + to +++ (table 1).

2. Twelve animals were given intravenous injections of dried clumps of BCG. These clumps tended to lodge in the lungs and produce a few nonprogressive tubercles. These twelve allergic animals and twelve normal animals were then given subcutaneous injections, as already described, of 0.01 mg. of the Ravenel bovine strain. All the animals were killed in forty-five days. A comparison of the degrees of tuberculosis in the normal animals and in the animals previously

given intravenous injections of dried living BCG is seen in table 2. All the normal animals had tuberculosis in the lungs of grades ++ to ++++. All but five of these twelve normal animals had tuberculosis in the kidneys of grades + to ++++.

TABLE 1.—*Degree of Tuberculosis Forty-Five Days After Subcutaneous Injection of 0.01 Mg. of a Virulent Bovine Strain (Ravenel) into Normal Rabbits and Rabbits Previously Made Allergic with a Subcutaneous Injection of Living BCG*

	Normal Rabbits		Allergic Rabbits		
Number	Lungs	Kidneys	Lungs	Kidneys	Mantoux Reaction
1	++	0	0	0	+
2	+++	0	0	0	+
3	+++	0	0	0	+
4	+++	+	0	0	++
5	+++	+	0	0	++
6	+++	+	0	0	++
7	+++	+	0	0	++
8	+++	+++	0	0	+++
9	+++	+++	0	0	+++
10	++++	+++	0	0	+++
11	++++	+++	0	0	+++
12	++++	+++	0	0	+++
13	++++	++++	0	0	+++
14	++++	++++	0	0	+++
15	++++	++++	0	0	+++
16	++++	++++	0	0	+++
17	.....	.....	0	0	+++

TABLE 2.—*Degree of Tuberculosis Forty-Five Days After Injection of 0.01 Mg. of a Virulent Bovine Strain (Ravenel) into Normal Rabbits and Rabbits Previously (Two Months Before) Given Intravenous Injections of Dried Clumps (Four to Five) of Living BCG*

	Normal Rabbits		Rabbits Previously Given Injection of BCG	
Number	Lungs	Kidneys	Lungs	Kidneys
1	++	0	0	0
2	++	0	0	0
3	++	0	0	0
4	+++	0	0	0
5	+++	0	0	0
6	+++	+	0	0
7	+++	+	0	0
8	++++	++	0	0
9	++++	++	0	0
10	++++	++++	+	0
11	++++	++++	++	0
12	++++	++++	++	0

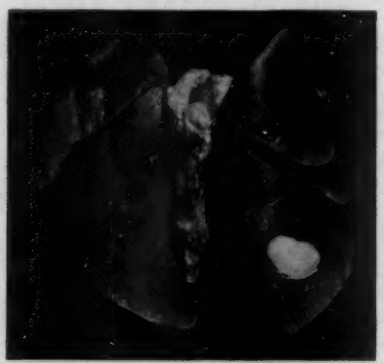
Three of the allergic animals had tuberculosis in the lungs—of grade + in one rabbit and grade ++ in two. None of the twelve allergic animals had tuberculosis in the kidneys.

3. Twelve animals were made allergic by injection of living BCG into the lung through a long needle in the trachea. Local lesions in the lungs were produced with a fair degree of uniformity with this method. Forty-five days after these twelve allergic animals and twelve normal animals had been given injections of

the virulent bovine strain, all the animals were killed and the degrees of tuberculosis noted (table 3). All the twelve normal animals had tuberculosis (grades ++ to ++++) in the lungs, and all but five had tuberculosis (grades + to ++++) in the kidneys. Two of the allergic animals had tuberculosis in the lungs, each of grade ++. None of the allergic animals had tuberculosis of any degree in the kidneys.

TABLE 3.—*Degree of Tuberculosis Forty-Five Days After Injection of 0.01 Mg. of a Virulent Bovine Strain (Ravenel) Subcutaneously into Normal Rabbits and Rabbits Previously Given Intrapulmonary Injections of BCG Through the Trachea*

	Normal Rabbits		Rabbits Previously Given Intrapulmonary Injections of BCG	
Number	Lungs	Kidneys	Lungs	Kidneys
1	++	0	0	0
2	++	0	0	0
3	++	0	0	0
4	+++	0	0	0
5	+++	0	0	0
6	+++	+	0	0
7	++++	+	0	0
8	++++	++	0	0
9	++++	++	0	0
10	++++	++++	0	0
11	++++	++++	++	0
12	++++	++++	++	0



Photograph showing an experimentally produced Ghon tubercle in the lower lobe of the right lung of a rabbit.

4. Twelve animals in the group were made allergic by injection of living BCG into the lung through the thoracic wall and pleura. This method proved to be the most constant in producing Ghon tubercles. Nodular areas could be produced in the lungs in practically all the animals. Well developed tubercles were observed in about two weeks. A Ghon tubercle produced by this method is shown in the figure.

The degree of allergy which developed in the animals ranged from + to +++. These twelve animals, with twelve normal animals, were then given subcutaneous

injections of 0.01 mg. of the Ravenel bovine strain. All the animals were killed in forty-five days. The degrees of tuberculosis at the points of subcutaneous injection, in the lungs and in the kidneys, were noted (table 4). In all the normal animals tuberculosis was present at the point of subcutaneous injection of the virulent strain, in grades of from ++ to ++++. Tuberculosis was present in the lungs of all the animals in grades of from ++ to ++++. In the kidneys of six of the twelve normal animals, tuberculosis was present in grades of from + to ++++. Tuberculosis was noted at the point of subcutaneous injection of the virulent strain in only three of the allergic animals, in each instance as grade +. No tuberculosis was seen in the lungs or kidneys of any of these twelve allergic animals.

The time at which allergy appears in the development of tuberculous lesions is often not taken into account by persons who consider allergy

TABLE 4.—*Degree of Tuberculosis Forty-Five Days After Injection of 0.01 Mg. of a Virulent Bovine Strain (Ravenel) Subcutaneously into Normal Rabbits and Rabbits with an Experimental Ghon Tubercle (Intrapleural) and Allergy (+ to +++)*

	Normal Rabbits			Rabbits with Ghon Tubercle		
Number	Subcutaneous	Lungs	Kidneys	Subcutaneous	Lungs	Kidneys
1	++	++	0	0	0	0
2	++	++	0	0	0	0
3	+++	++	0	0	0	0
4	++++	++	0	0	0	0
5	++++	+++	0	0	0	0
6	++++	+++	0	0	0	0
7	++++	+++	+	0	0	0
8	+++	++++	+	0	0	0
9	++++	++++	++	0	0	0
10	++++	++++	++	0	0	0
11	++++	++++	++++	+	0	0
12	++++	++++	++++	+	0	0
13	++++	++++	++++	+	0	0

to be the chief factor responsible for the clinical difference between primary and secondary pulmonary tuberculosis.

Myers,[2] speaking of allergy due to the primary pulmonary infection, stated: "It precedes x-ray evidence by weeks and months and in many cases it remains the sole evidence of tubercle formation." Dienes and Mallory [11] reported that allergy may occur in guinea-pigs as early as three days after infection. Rothschild, Friedenwald and Bernstein noted the occurrence of allergy seven days after infection.

In my previous experiments [12] in which allergy was produced in rabbits by subcutaneous or intrapulmonary injection of living BCG, I found that allergy was regularly present in three weeks and that if it had not occurred by that time it did not appear.

11. Dienes, L., and Mallory, T. B.: Am. J. Path. **8**:689, 1932.
12. Clawson, B. J.: Arch. Path. **20**:343, 1935.

The time at which allergy appeared in animals in which experimental Ghon tubercles were produced is shown in table 5.

Thirty-nine animals in which the Ghon tubercle was due to an intrapulmonary injection of BCG were tested for allergy at intervals with an intracutaneous injection of 1 mg. of old tuberculin. One animal was tested six days after the intrapulmonary injection and showed no allergy. Allergy of grade + appeared in one animal in eleven days, of grades + to +++ in sixteen animals in fourteen days, and of grades + + to + + + in twenty-one animals in twenty-one days. As in the animals which were given subcutaneous injections, allergy could be counted on to appear in three weeks.

To determine whether allergy appeared as early as in the group just reported on when the allergy-producing lesion in the lung was due to a small amount of infection with a virulent strain, thirteen animals were given injections by the intra-

TABLE 5.—*Time of Appearance of Allergy After Intrapulmonary Inoculation of Rabbits with 2 Mg. of BCG*

Number of Rabbits Inoculated	Period Before Appearance of Allergy, Days	Degree of Allergy
1	6	0
1	11	+
16	14	+ to +++
21	21	++ to +++

TABLE 6.—*Time of Appearance of Allergy After Intrapulmonary Inoculation of Rabbits with 0.001 Mg. of a Virulent Bovine Strain*

Number of Rabbits Inoculated	Period Before Appearance of Allergy, Days	Degree of Allergy
1	20	0
6	20	+
5	20	++
1	20	+++

pulmonary method of 0.001 mg. of a virulent strain of bovine tubercle bacilli and were tested for allergy as usual (table 6). The observations were made twenty days after the intrapulmonary injection of the virulent organisms. One animal showed no allergy; six had allergy of grade +, five of grade ++ and one of grade +++.

From the observations on patients and animals it seems evident that allergy appears early in the development of a tubercle. Cases of human tuberculosis are probably never seen in which there is not or has not been allergy. Of course, the allergy may disappear temporarily under various conditions. The person having a Ghon tubercle is in the allergic state early, and the course of development of the lesion is under the influence of allergy as truly as is the secondary or adult type of infection.

COMMENT

A series of experiments is described in which the combined effects of allergy and resistance on the pathogenesis of pulmonary tuberculosis in the rabbit were studied. With the exception of the first group of animals in which the allergy was produced by a subcutaneous injection of living BCG, the allergy and resistance were due to lesions in the lungs brought about by injection of living BCG. The BCG strain was used in producing the lesions in the lungs so that nonprogressive tubercles would develop. The purpose in producing nonprogressive lesions in the lungs was to have a condition in the rabbit similar, as nearly as possible, to the allergic and immune states in a person with a healed or retarded Ghon tubercle.

Four methods were used in bringing about the allergic and immune states: (1) subcutaneous injection of living BCG; (2) intravenous injection of dried clumps of BCG; (3) intrapulmonary injection of BCG through a long needle in the trachea and (4) intrapulmonary injection of BCG through the thoracic wall and pleura. The last method proved the most efficient in producing localized nonprogressive lesions in the lungs. In nearly all instances these experimentally produced Ghon tubercles were in the upper part of the lower lobe of the right lung.

In previous experiments it was suggested that allergy may have an ill effect on the lung, since with subsequent infection greater necrosis tends to occur in a shorter time than that in normal animals inoculated with an equal dose of bacilli. The present experiments definitely showed, however, that even though allergy was present with lesions in the lungs, the associated immunity was great enough to overbalance the ill effects of the allergy and, in addition, to give a marked degree of protection.

My purpose in the experiments was not to recommend a method of vaccination against tuberculosis but to suggest, on the basis of a comparative study, the prognosis of pulmonary tuberculosis from the standpoint of the influence of the factors of allergy and resistance in a person with a healed or an arrested Ghon tubercle. The comparison is not exact, since the rabbit and man react somewhat differently to tuberculous infection, but the advantage seems to be on the side of the human subject, since human tuberculosis shows a much greater tendency to arrest and healing than rabbit tuberculosis.

The experiments showed that rabbits which were allergic had a marked degree of resistance to subsequent infection with a virulent strain. This resistance was not dependent on allergy alone, since in previous experiments it was found that resistance could be developed as well by a method in which allergy did not occur. It was also found that allergy could disappear to the extent that no positive cutaneous

reaction was elicited by an intracutaneous injection of 1 mg. of old tuberculin, and yet the animals could have a high degree of resistance against virulent infection.

The fact that allergy occurs so early in the development of tubercles does not support the theory that the factor causing the severe secondary or adult type of pulmonary tuberculosis is allergy, for evidently all tuberculosis, both the childhood and the adult type, even before it can be observed anatomically or clinically, is associated with and influenced by allergy.

The experiments definitely appeared to indicate in a comparative way that, while it is true that most persons who die of tuberculosis have had an earlier primary pulmonary infection, the factor in bringing about the more extensive secondary tuberculosis is not allergy. The experiments also demonstrated that a marked degree of resistance, even in the presence of allergy, may be developed with primary pulmonary infection (Ghon tubercle).

SUMMARY

Animals having a Ghon tubercle with allergy may show a marked degree of resistance against further tuberculous infection.

Some factor or factors other than allergy should be sought to account for the more extensive secondary or adult type of pulmonary tuberculosis which may occur in patients who already have a nonprogressive Ghon tubercle.

Laboratory Methods and Technical Notes

FLUORESCENT MICROSCOPY

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Ultraviolet radiation has been used advantageously in the microscopic examination of living and dead tissues.[1] In recent years, it has also been applied in conjunction with microscopic studies (fluorescent microscopy). Such histologic examinations of animal and human tissues have been reported by Hamperl and others.[2] I have been interested in this phase of the subject, especially in regard to its practical diagnostic value.

METHOD USED IN EXAMINATION OF TISSUES

Duplicate microtome-cut sections (from 7 to 8 microns in thickness) were prepared from tissues obtained at operations and in the postmortem examination room. The tissues had been embedded in paraffin after having been given routine treatment. One set of sections was fastened to ordinary glass slides and stained with hematoxylin and eosin. The duplicate set of sections was attached by means of egg albumin to special slides which permit the penetration of ultraviolet radiation (these are known as U-V slides). The sections placed on the latter slides were neither protected with cover-slips nor stained. Prior to examination with the fluorescent microscope, the sections were immersed in xylene for approximately one-half hour. The sections were then dried in the air at room temperature. After such treatment, the attached sections remained still satisfactory for examination, even months later. In conjunction with such paraffin sections, frozen sections were occasionally prepared.

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From the Laboratory Division, Hospital for Joint Diseases.

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1. Sutro, C. J., and Burman, M. J.: Arch. Path. **16**:346, 1933.

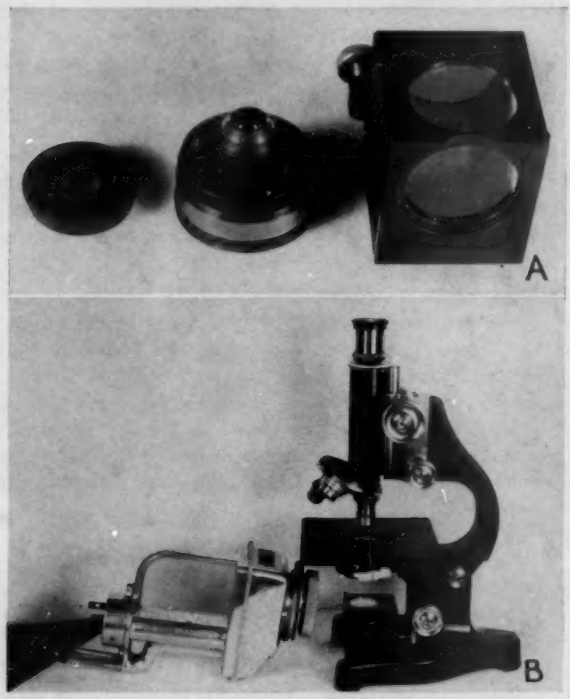
2. Kohler: Ztschr. f. wissensch. Mikr. **21**:129 and 273, 1904. Walkhoff: Sitzungsb. d. Gesellsch. f. Morphol. u. Physiol. in München **33**:7, 1922; Verhandl. d. phys.-med. Gesellsch. **49**:159, 1924. Konig: ibid. **49**:160, 1924. Bommer, S.: Acta dermat.-venereol. **10**:390, 1929. Lucas, F. F.: Science **71**:515, 1930; Proc. Nat. Acad. Sc. **16**:599, 1930. Hartoch, W.: Ztschr. f. d. ges. exper. Med. **79**: 538, 1931. Singer, E.: Science **75**:289, 1932. Thenon, J., and Pirosky, I.: Rev. Soc. argent. de biol. **8**:201, 1932. Erös, G.: Centralbl. f. allg. Path. u. path. Anat. **54**:385, 1932. Fowler, E. P., Jr., and Applebaum, E.: Anat. Rec. **55**:23, 1932. Radley, J. A., and Grant, J.: Fluorescence Analysis in Ultra-Violet Light, New York, D. Van Nostrand Company, 1933. Bommer, S.: Dermat. Ztschr. **67**:319, 1933. Exner, R.: Psychiat.-neurol. Wchnschr. **35**:319, 1933; **36**:291, 1934. Querner, F.: Ztschr. f. mikr.-anat. Forsch. **32**:444, 1933. Baroni, B.: Arch. ital. di dermat., sif. **9**:543, 1933. Haitinger, M., and Hamperl, H.: Ztschr. f. mikr.-anat. Forsch. **33**:193, 1933. Hamperl, H.: Virchows Arch. f. path. Anat. **292**:1, 1934; Arch. Path. **19**:838, 1935.

APPARATUS

For the execution of fluorescent microscopy, the following parts are required:

1. An ordinary microscope
2. A water-cooled quartz mercury vapor arc lamp
3. A Corex ultraviolet ray filter
4. A quartz prism reflector
5. A quartz condenser (Abbe type without the iris diaphragm)
6. A special eyepiece cap filter.

The apparatus as assembled for use is shown in the accompanying figure. All examinations were done in a completely darkened room.



A, from left to right, the special eyepiece filter, the quartz condenser and the quartz prism reflector. *B*, the water-cooled quartz mercury vapor arc lamp and the parts assembled in the microscope.

OBSERVATIONS

It has been demonstrated that most tissues when examined under filtered ultraviolet radiation will fluoresce. The colors depend for the most part on the chemical constituents of the tissues. The chemical constitution in turn is partly dependent on the condition of the specimen (well preserved or dried) and on the age of the person from whom it was obtained. It is recognized that secondary or false fluorescence may be produced by substances used in the preservation or in the embedding of the tissues. It has been found also that a diluted solution of

formaldehyde U.S.P. (1:10) used for fixation and paraffin used for embedding interfere least with the natural fluorescence of tissues.

On the basis of the examination of hundreds of sections, it may be said that differentiation of some of the constituents of tissue is possible by fluorescent microscopy. For example, the examination of an unstained paraffin-embedded section of human spleen revealed a purplish fluorescence of the capsule as compared with a deep brown fluorescence of the pulp. The cells of the islands of Langerhans fluoresced reddish as compared with a dark brown tint of the excretory portions of pancreatic tissue. Small foci of pigment were noted by fluorescence in the stroma of the mucosa of the gallbladder and intestine, in the connective tissue and muscle fibers of the heart, and in the stroma and parenchyma of many other organs. The nature of these pigments is far from established. They have been considered by pathologists to be the result of wear and tear.[3]

It was also found that paraffin-embedded sections of decalcified lamellar bone may fluoresce one of several shades varying from blue to gray. Five per cent solution of nitric acid was used for decalcification. The degree of decalcification, the age of the subject when the bone was obtained, and the thickness of the prepared paraffin sections were some of the main determinants of variations in the color. Bone marrow fluoresced from brown to brownish yellow. Isolated foci of deep brown and golden yellow pigment were occasionally noted in them. It is of interest that in several instances the contents of arterial channels entering lymphoid marrow fluoresced differently from the contiguous marrow tissue. This in several instances permitted recognition of the smaller arterial channels.

It may be said that fluorescent microscopy of tissues aids in the detection of certain pigments, elastic tissues and lipoids. Fluorescent microscopy may be considered as a spectroscopic examination. The chemical identification of each specific color as seen by fluorescent microscopy is open to further investigation. Histologic diagnoses cannot be based on fluorescent microscopy alone. The method has limited practical value. It should be applied particularly in those cases in which depositions of abnormal pigments in tissue are suspected.

In addition to the aforementioned examination of human tissues, an investigation is also reported concerning the advantages of fluorescent microscopy in an experimental study. Specifically, examination was made of the musculoskeletal system and teeth of laboratory animals in which the distal segments of the common bile ducts were ligated and severed.

Young and adult rats and cats were used. The operations were performed under sodium amytal anesthesia; strict surgical asepsis was maintained. Some of the animals died within a week; others were killed at intervals of from four to seven days over a period of three weeks. Control animals were killed at similar intervals. The costochondral junctions, symphyses pubes, vertebrae, tibias, femurs and lower incisors were examined. They were preserved in a diluted solution of formaldehyde U.S.P. (1:10). The hard tissues were decalcified in a 5 per cent solution of nitric acid. The tissues were prepared in the routine manner for

3. Lubarsch, O.: Virchows Arch. f. path. Anat. **239**:491, 1922.

embedding in paraffin. Duplicate sections were cut. One set was placed on special U-V glass slides; the other, on ordinary glass slides. The latter only was stained with hematein and eosin.

Fluorescent microscopy of the unstained paraffin-embedded sections of the tissues of these animals revealed many points of interest, some of which were not noted in the corresponding sections stained with hematein and eosin. The innermost layer of the periosteum of the long bones as well as the walls of the canals of the larger blood vessels in the cortices fluoresced yellow. The cortices, however, fluoresced grayish purple. The cortical lamellae situated very close to the canals of the large vessels were in some cases of a lighter purplish color.[4]

The cartilaginous matrix in the costochondral junctions showed a sharp differentiation in color just at the provisional zone of calcification. The cytoplasm of the cartilage cells in the proliferating zones was yellow. This coloration was occasionally noted in the cytoplasm of the cells in the hypertrophic and resting layers. (Since these sections of tissue were immersed in alcohol during the process of embedding, one may assume that the intense yellow color was not due to the presence of neutral fat.[5] Some parts of the cartilaginous matrix fluoresced a brownish-purple tint, indicating, perhaps, a mixture of golden yellow and purple. In the provisional zone of calcification, the cartilage cores were surrounded for the most part by a narrow zone of brown (new young bone) and an outer wider layer of yellow (marrow). Scattered small areas of yellow were occasionally encountered in the matrix and in the cartilage cell lacunae of the articular cartilages of the long bones. When present, these were seen directly beneath the articulating surface.

The fibrocartilage of the vertebrae and that of the symphysis pubis showed no yellow, but the walls of blood vessels situated in the fibrocartilage or in the fibrous ligaments fluoresced yellow. The nucleus pulposus and the spinal cord fluoresced yellow.

Examination of the incisors of the rats revealed that the pulp, the acalcified enamel, and the odontoblastic and ameloblastic layers also fluoresced yellow. The dentin, however, was purple.

The yellowish fluorescence of the tissues described is directly related to the surgically induced icterus, for it was not noted in the sections of tissues obtained from the control animals. The corresponding sections stained with hematein and eosin, from the experimental as well as from the control animals, did not reveal the generalized yellowish discoloration.

SUMMARY

Fluorescent microscopy aids in the detection of certain pigments deposited in tissues. Differentiation of the components of tissues without the use of stains is also possible. However, a histologic diagnosis cannot be made by this method alone. In experimental animals, for example, rats or cats in which the common bile duct had been ligated and severed, fluorescent microscopy revealed many interesting changes which were not seen in the sections stained with hematein and eosin.

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4. Schmorl, G.: Virchows Arch. f. path. Anat. **275**:13, 1930. Fikentscher, R.; Fink, H., and Emminger, E.: ibid. **287**:764, 1933. Emminger, E., and Büchele, B.: ibid. **295**:46, 1935.

5. Putschar, W.: Beitr. z. path. Anat. u. z. allg. Path. **87**:526, 1931.

Case Reports

PULMONARY EMBOLUS CONTAINING CEREBRAL TISSUE

CECIL KRAKOWER, M.D., Boston

There are few reports dealing with pulmonary emboli made up in great part of cerebral tissue. Textbooks refer to parenchymatous emboli but do not specifically mention nerve tissue. Ceelen [1] states that *"embolische Verschleppungen von Gehirnsubstanz sind zweifellos sehr grosse Seltenheiten"* (embolic mislayings of brain substance are doubtless very great rarities) and refers to reports by Merkel, Walcher, Askanazy and Hückel. In view of the observations in the present case this brief account seems warranted.

A boy aged 23 months fell from an unfinished staircase about 12 feet (about 365 cm.) to a cement floor. He was unconscious for a short period and subsequently remained drowsy and was irritable when disturbed. On his admission to the hospital the following morning, the chief findings were: upward rolling of the eyes, rotation of the head to the right, spasticity of the lower limbs with hyperactive reflexes, Kernig's sign and bloody cerebrospinal fluid. The pupils were equal and regular. The fundi could not be seen. Roentgen examination disclosed widely separated fracture lines extending transversely across both parietal bones, with stellate arrangement and overlapping in the left parietal region. As right hemiplegia developed during the morning following admission, an exploratory operation was undertaken, and the depressed bony fragments were elevated. His condition rapidly grew worse. The pulse became irregular, and air hunger developed. He died that afternoon, two days after the injury.

The pertinent observations at autopsy were: comminuted fracture of the left parietal bone with extensive laceration of the dura, herniation and laceration of the underlying cerebral tissue over an area 2.5 by 1.5 cm., involving chiefly the left precentral and postcentral gyri; rupture of the superior longitudinal sinus and presence of a massive adherent clot; complete thrombosis of the sinus anterior to the rupture, and extensive subdural and epidural hemorrhages with transverse and longitudinal linear fractures of the right parietal bone.

The lungs externally were not remarkable except for dependent congestion and generalized fine nodularity to palpation throughout. The main pulmonary arterial trunks were free from thrombi. Sections of the lungs were characterized by the presence of numerous thrombi plugging the larger arterial radicles, associated here and there with larger and smaller dark red hemorrhagic areas. In the upper part of the right middle lobe these areas assumed a single large wedge shape. Microscopically in one of the larger arterial vessels there was a central mass of cerebral tissue (figs. 1 and 2) with a granular, ground-glass-appearing substrate

From the Department of Pathology, Harvard Medical School and the Children's Hospital.

1. Ceelen, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1931, vol. 3, p. 107.

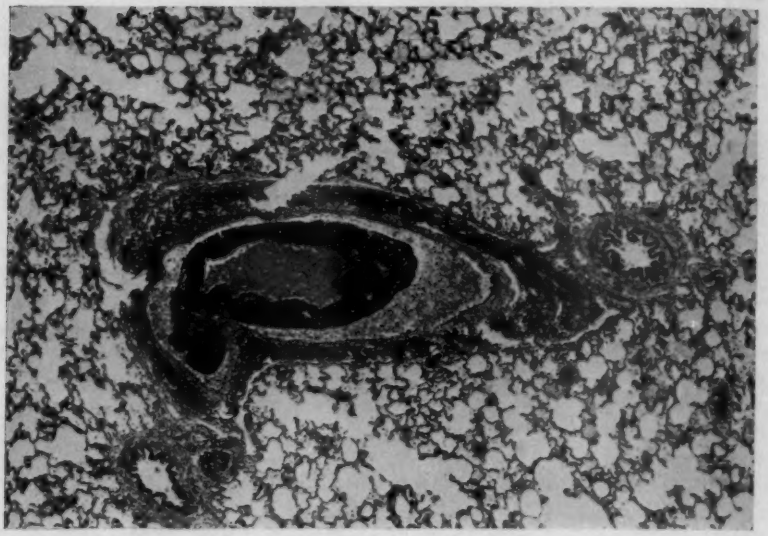

Fig. 1.—Pulmonary artery with an embolus containing cerebral tissue; reduced from a low magnification; stained with Mallory's phosphotungstic acid hematoxylin. Note the surrounding layer of dense laminated fibrin. The defect in the upper part of the wall of the vessel is an artefact.

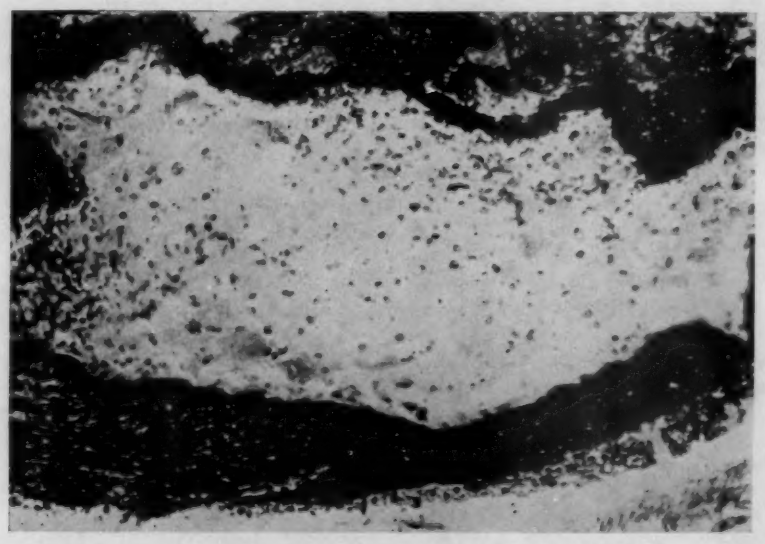

Fig. 2.—A high magnification of the embolus shown in figure 1. Note the swollen glia cells and the intact capillaries.

and fairly regularly placed swollen glia cells. Stained with Mallory's phosphotungstic acid hematoxylin, the substrate presented deep blue stipplings within a light bluish background. Patent capillaries were here and there recognizable, and there were scattered red blood cells throughout the tissue. At the periphery and in part infiltrating it, there were large cells with granular cytoplasm, occasionally lightly vacuolated, with, at times, eccentrically placed small deep-staining nuclei strongly resembling *Gitterzellen,* or compound granular corpuscles. The whole mass was encircled by closely packed laminated fibrin with scattered platelet aggregates. At one point this was in contact with the denuded intimal surface of the vessel wall. Elsewhere, separating the embolus from the endothelial-lined surface, were red blood cells with here and there some fibrin and platelets. The other emboli in sections studied microscopically were made up of blood elements only.

In the absence of recognizable nerve cells, it is presumed that the cerebral tissue within the embolus is from the white matter. The fact that there are intact patent capillaries with lining endothelial cells and well preserved red blood cells presupposes recent attachment to and connection with the rest of the brain substance. The presence of a number of cells resembling compound granular corpuscles, skirting the periphery indicates that it was situated in a traumatized and reactive zone from which it might readily have been dislodged. Furthermore, it may reasonably be supposed that subsequently, with repeated intracranial bleeding, it was completely detached and lodged in the torn dural sinus. There it was encircled by platelets and fibrin and probably enmeshed in the thrombus occluding the proximal torn end of the vessel. It was finally released into the general circulation some time shortly before death, possibly during the operation.

News and Notes

University News, Promotions, Resignations, Appointments, Deaths, etc.—Cyril N. H. Long, director of the Cox Medical Institute of the University of Pennsylvania, has been appointed professor of physiologic chemistry at Yale University, succeeding the late Lafayette B. Mendel.

The Charles Mickle Fellowship of the University of Toronto has been awarded to Donald D. Van Slyke, of the Rockefeller Institute for Medical Research, in recognition of his work in biologic chemistry.

The Sigma Xi Semi-Centennial Research Prize in the biologic sciences has been awarded to Richard E. Shope, of the Rockefeller Institute for Medical Research, for his work on the etiology of swine influenza.

The gold medal of the American Society of Clinical Pathologists has been awarded to Rigney D'Aunoy and Emerich von Hamm, of the Louisiana State University Medical Center, for their work on lymphogranuloma venereum.

The George M. Kober medal of the American Association of Physicians has been awarded to A. R. Baldwin of Saranac, N. Y., for his work on tuberculosis.

The James E. Stacey Award for 1936 of the University of Cincinnati Medical College has been given to Bernard L. Wyatt, of Tucson, Arizona, for his work in the field of focal infection.

John L. Jacobs has been appointed associate professor of pathology and bacteriology in Tufts College Medical School, Boston.

In New York University, Irving Graef has been promoted to the position of associate professor of pathology and Harrison S. Martland to that of professor of forensic medicine.

Frederick F. Russell, formerly director of the International Health Division of the Rockefeller Foundation, has been awarded the Public Welfare Medal of the National Academy of Sciences.

David A. Wood has been promoted to the position of associate professor of pathology and Alvin J. Cox Jr. to that of assistant professor of pathology in the Stanford University School of Medicine.

Alice Hamilton, who until recently occupied a professorship of industrial medicine in the school of public health of Harvard University, has been awarded the gold medal of the Chi Omega Sorority for her work in industrial pathology.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

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Pathologic Anatomy

Pathologic Changes in Asthmatic Infants. G. L. Waldbott, Am. J. Dis. Child. **49**:1531, 1935.

Death from asthma among young children and infants is rare. Two cases are described in which asthma had been present for three weeks and six weeks, respectively. The literature does not reveal a report of autopsy in any case in which there was so short a duration. There is a distinct difference between the pulmonary observations in infants and those in adults. In infants the following manifestations are noted: edematous hemorrhagic areas in the lungs, prevalence of lymphocytic rather than of leukocytic infiltration, absence of evidence of a marked degree of bronchospasm and of emphysema and lack of a material amount of mucus in the bronchi. These changes resemble more closely those observed in the lung of an anaphylactic dog than those in the lung of an asthmatic adult. These observations tend to substantiate the theory that anaphylaxis and atopy are identical, as they indicate that asthma in its early form is identical with anaphylactic shock. The pathologic observations in an asthmatic adult may then be interpreted as being representative of a protective physiologic set-up which may be the result of a more prolonged tendency to counteract the shocking influence of the antigen toward which sensitivity exists. So-called thymic death is decidedly more prevalent among infants than death from asthma, as indicated by the existing literature and hospital records. Considering previous pathologic evidence of the identity of so-called thymic death and allergic shock, which I have presented elsewhere, it seems possible that thymic death of infants may be the equivalent of death from asthma.

From the Author's Summary.

Late Infantile Amaurotic Idiocy with Marked Cerebral Atrophy. R. Richter and A. H. Parmelee, Am. J. Dis. Child. **50**:111, 1935.

The pathogenesis of amaurotic idiocy has long been a source of dispute and theorizing. The brain of our patient offers evidence pertinent to some points of this problem. Most contemporary writers have been in agreement that the disease is degenerative rather than developmental so far as the alterations in the ganglion cells are concerned, but it has been thought by many that when defects of white matter are present these are to be explained as being due to arrested development, that is, to aplasia in the true sense of the word. Thus, Savini-Castano and Savini concluded that in their case they were dealing with a degenerative process in an underdeveloped brain. But in our case the extreme disappearance of cerebral white matter is in itself indicative of the degenerative character of the changes, for it is inconceivable that any great part of the process could be on the basis of aplasia, considering that the child was as nearly normal as he was until 14 months of age. Brodmann's case forms an even stronger argument than ours in this direction. In his patient the symptoms did not begin before the child was 4 years of age; still there was cerebral atrophy almost equal to that in the brain of our patient. One of the chief objections to regarding the changes in the white matter as degenerative is the relative absence of fat granule cells. However, cases have been recorded in which such cells presented the outstanding glial change, notably Globus' first case. If the disease is looked on as a disturbance of the trophic metabolism of the cell, as, for example, in Marinesco's ingenious speculation on the absence of oxidizing ferments, rather than as primary damage of the conduction mechanism, this will

explain the apparently normal period clinically. It is likely that the pathogenic factors are active even in the fetal period and thus lead to what might be termed acquired aplasia. What proportion of the changes are on this basis and what is the result of the degeneration of fully or partially developed structures cannot be decided. This must vary greatly from case to case.

Involvement of the white matter in amaurotic idiocy is a result of primary participation of the glia in the endogenous degenerative process. It is not possible to separate cases of amaurotic idiocy into well defined and constant subgroups. On the other hand, all the cases taken together form a firm nosologic unit, clinically and anatomically. Retinitis pigmentosa may be a feature of cases of amaurotic idiocy which otherwise clinically exhibit the characteristics of the infantile form. All the retrogressive pathologic changes are the expression of an acquired degenerative process.

FROM THE AUTHORS' SUMMARY.

THE INFLUENCE OF ANAPHYLACTIC SHOCK ON THE FINER STRUCTURE OF THE LIVER IN THE DOG. H. L. WEATHERFORD, Am. J. Path. **11**:611, 1935.

Anaphylactic shock in the dog presents two characteristic stages: *(a)* a primary shock, usually of short duration, and *(b)* a secondary shock, more severe and prolonged. An extreme congestion of the liver, a marked fall in arterial blood pressure and an increase in the flow of lymph from the thoracic duct are displayed as features of primary shock. Some swelling of the hepatic cells appears at the centers of the lobules. Secondary shock produces more damage to the parenchyma of the liver, such as "cloudy swelling," hydrops, vacuolation of the cytoplasm and finally central necrosis. Accompanying these degenerative changes there is found a diminution in caliber of the sinusoids of the hepatic lobules, also of the efferent veins of the liver. A stasis of blood in the narrowed sinusoids leads to formation of hyaline plugs or thrombi. A disorganization of the sinusoidal endothelium, proliferation of the endothelial cells and increased phagocytosis by the Kupffer cells are very evident. The taking up of whole erythrocytes by the parenchymatous cells of the liver is noted in severe secondary shock. Both the hepatic cells and the Kupffer cells reveal an increment of iron. The chondriosomes reflect the extent of the parenchymatous injury. In the centers of the lobules spheric chondriosomes abound. Later, in some of the most central cells, chondriolysis occurs accompanying karyorrhexis and karyolysis. Homogeneous atrophy preceded by chondriomegaly appears in certain cells or groups of cells in the midzone and peripheral zone of the hepatic lobule. Two forms of "dark cells" may be differentiated on the basis of nuclear and cytoplasmic appearances.

FROM THE AUTHOR'S SUMMARY.

CROSSED ATROPHY OF THE CEREBELLUM. G. B. HASSIN, Arch. Neurol. & Psychiat. **33**:917, 1935.

Crossed cerebellar atrophy is a combination of atrophy of a lateral cerebellar lobe with a destructive lesion of a contralateral cerebral hemisphere. The clinical picture is that not of a cerebellar but of a cerebral disease. This was the case in a woman, aged 28, who, aside from cardiac and pulmonic complications, presented a coarse tremor and deformity of the right upper extremity with a decreased muscle tone. Necropsy revealed, among visceral changes, atrophy of the entire left cerebral hemisphere, of the frontal lobe of the right hemisphere and of the right cerebellar lateral lobe. The left frontal and occipital lobes, including the basal ganglions, pons and medulla, were especially involved. There was complete degeneration of the Purkinje cells of some cerebellar lamellae, and of the molecular and granular layers and their replacement by glia tissue, as well as partial and scattered degeneration of the white substance of the cerebellum, of the pons (mainly the right half) and of the ganglion cells of the contralateral cerebral hemisphere, especially of the motor center for the arm. The degeneration extended from the cerebrum to the cerebellum along the corticopontile and pontocerebellar pathways; that is, it was primary in the cerebral cortex and involved secondarily the pons and the cerebellum.

FROM THE AUTHOR'S ABSTRACT.

EXTENSIVE CALCIFICATION IN THE BRAIN. J. KASANIN and R. P. CRANK, Arch. Neurol. & Psychiat. **34**:164, 1935.

The authors describe extensive calcification of the capillaries and arteries in a man, aged 32, who for the last ten years of his life showed marked mental changes with signs of extrapyramidal lesions and convulsions. Necropsy revealed large deposits of calcium salts in both the basal ganglions and calcification of the capillaries and arteries of the cerebellar and cerebral hemispheres beginning with the second cortical layer and of their white substance, midbrain and pons. The veins were practically unaffected. The calcification was due to deposits of lime salts in the adventitia, from which it extended to the media, obliterating the Virchow-Robin spaces. In addition to true calcification there were also deposits of small quantities of iron. It is thought that the deposition of iron preceded that of calcium, as in some less advanced cases pathologic iron was present without calcium. The condition, which was diagnosed by roentgenography, was also found in a sister of the patient.

GEORGE B. HASSIN.

Pathologic Chemistry and Physics

SPECTROGRAPHIC STUDY OF LEPROUS LESIONS. E. V. COWDRY, L. F. HEIMBURGER and P. S. WILLIAMS, Am. J. Path. **12**:13, 1936.

The potassium-calcium ratios determined for leprous skin from five lepers are on the average probably three times those obtained for normal skin from persons of the same age group. The sodium-calcium, magnesium-calcium and iron-calcium ratios show no notable variations from the normal. A fair correlation is obtained between the potassium-calcium ratio with known duration of the disease and the volume of leprous cells in the tissue analyzed spectrographically. It may be conditioned by increase in potassium or decrease in calcium but probably by both. Histospectrography as developed by Scott and his collaborators can evidently be used for the study of small pieces of tissue removed at biopsy which would be altogether insufficient for routine chemical analysis by ordinary methods. Once the spectrograms have been taken, essentially the same procedure is employed for the determination of ratios between several elements, whereas the chemical estimation of each element would be different and in some cases very involved.

FROM THE AUTHORS' SUMMARY.

CHEMICAL ANALYSIS OF ATHEROSCLEROTIC LESIONS IN THE HUMAN AORTA. P. M. ZEEK, Am. J. Path. **12**:115, 1936.

Samples from normal and atheromatous areas of aortas obtained at autopsies on eleven adults were analyzed quantitatively for lipids and the results compared with microscopic sections from the same areas. It was found that the morphologic increase in lipids in the aorta during the progress of atherosclerosis is accompanied by a corresponding chemical increase in lipids, including total cholesterol, free cholesterol, cholesterol esters, fatty acids, lecithin and total lipid. The ratio of free cholesterol to ester cholesterol decreases during the early stages of the process but shows a marked increase in the advanced stages. This confirms the conclusions of Meeker and Jobling but is contrary to that of Schönheimer.

FROM THE AUTHOR'S SUMMARY.

CLINICAL SPECTROSCOPY. L. E. GAUL and A. H. STAUD, Arch. Dermat. & Syph. **30**:433, 1934.

Spectrograms of biopsy specimens from patients who had received silver arsphenamine were compared with those of standard solutions of silver nitrate. A total dose of silver arsphenamine varying from 0.225 to 0.9 Gm. gives a silver content slightly greater than that found in normal skin. The deposition of silver is uniform throughout the dermis, and retention by the connective tissue is cumulative

and directly proportional to the total dose received. The quantity remained proportional to the total dose up to two and one-half years after the drug had been taken. Argyria becomes clinically evident after a retention of silver approximately the equivalent of that contained in 8 Gm. of silver arsphenamine.

S. W. Becker.

The Plasma Lipids in Purpura Produced with Antiplatelet Serum. L. M. Tocantins and A. Cantarow, J. Immunol. **30**:261, 1936.

In the plasma lipids of dogs with thrombopenic purpura produced by injection of antiplatelet serum in moderate dosage the fatty acids show an immediate slight decrease followed from one to two days later by a marked increase, with a subsequent return to normal; there is an immediate increase in the phosphorus of these lipids that persists for from three to eight days; the cholesterol shows no significant changes.

From the Authors' Summary.

Anesthesia and Blood Lipids. E. M. Boyd, Surg., Gynec. & Obst. **62**:677, 1936.

The effect of nitrous oxide-ether anesthesia on the concentration of lipids in the plasma and in the erythrocytes was determined by oxidative micromethods. Within the first eight to ten hours lipopenia developed which was due to a decerase in the neutral fat and phospholipid of the erythrocytes and a fall in the neutral fat of the plasma. This was followed by lipemia about twenty-four hours after the administration of the anesthetic. The lipemia was due to a marked increase in the neutral fat, phospholipid and cholesterol esters of the red blood cells; the cholesterol fractions and phospholipid of the plasma were slightly decreased. From studies of the iodine number the plasma was found to contain more saturated phospholipids during the lipopenia and more unsaturated phospholipids during the lipemia, the erythrocytes discharging their saturated phospholipids in the lipopenic period. The neutral fat and cholesterol esters of the red cells became more unsaturated under the influence of anesthesia, while in the plasma no change occurred. The free cholesterol of the plasma increased during the lipopenic stage, while ester cholesterol did not, suggesting that anesthesia affects the metabolism of sterol independently of its effect through the intermediary of lipid metabolism. The results explain many of the discrepancies in earlier studies on man, and grouping earlier data according to species serves to bring into harmony certain discordant conclusions among studies on animals.

From the Author's Summary.

Microbiology and Parasitology

The Human Blood Group Factor. B. W. Fischer, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:136, 1935.

Group-specific anti-B immune serums were obtained by inoculation of sheep with human B red cells. To keep down the appearance of species-specific hemagglutinins it was necessary to bleed the animals soon after the series of injections were administered. The serums of the immunized sheep contained two agglutinins, one of which reacted only with human B red cells (anti-B_1) while the other reacted also with rabbit red cells (anti-B_2). Immune serums obtained by inoculating sheep with rabbit red cells contained only species-specific antirabbit antibodies but no anti-B_2 agglutinins.

I. Davidsohn.

The Serologic Differentiation of Two Species of Guinea-Pigs F. J. Holzer, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:170, 1935.

The common guinea-pig, Cavia porcellus, could be differentiated serologically from the Brazilian variety, Cavia rufescens. The common guinea-pig responded

to injections of the erythrocytes of the other variety with production of a specific lysin. Ox serum contains normal agglutinins for guinea-pig red cells. After absorption with the red cells of the common guinea-pig this serum still caused clumping of the red cells of the Brazilian variety. Inoculation of one species with the blood of the other failed to stimulate production of precipitins. Some of the common guinea-pigs became sensitized by injections of the blood serum of the Brazilian species and responded with a true anaphylactic shock to reinjection of the serum. The majority of the animals did not become sensitized.

I. DAVIDSOHN.

THE OCCURRENCE OF A WEAK BLOOD GROUP FACTOR IN AN ADULT. W. FISCHER and F. HAHN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:177, 1935.

A 33 year old patient with a clinical picture of septicemia was found to belong to group A, but the agglutinability of his erythrocytes was extremely low. Serum of group O agglutinated the cells in higher dilutions than did serum of group B or an antisheep serum. The serum had a very high titer of iso-agglutinins and of hetero-agglutinins as well as of heterohemolysins for sheep cells. The erythrocytes of this patient absorbed only very small amounts of iso-agglutinins from a serum of group O, but the absorbed antibodies could be readily separated from the red cells at 56 C. On the other hand, erythrocytes of the same blood group with a higher avidity for the corresponding iso-agglutinins gave up the attached antibodies only very sparingly. Fischer and Hahn emphasize the advisability of using serum of group O for blood typings in addition to serums of group A and B. This may help to demonstrate the proper blood group in erythrocytes with weak receptors.

I. DAVIDSOHN.

TRANSMISSION OF GONORRHEA TO CALVES. W. STOCKMAYER and J. SCHMITZ, Ztschr. f. Immunitätsforsch. u. exper. Therap. **84**:371, 1935.

When pus containing abundant gonococci from persons with recently acquired gonorrhea was rubbed into the vaginal mucosa of calves from 3 to 8 months old a mild local infection was produced in six of eleven animals. Hyperemia and a serous or mucopurulent exudate with occasional leukocytes, but mainly with desquamated epithelial cells, were present. Gram-negative diplococci, occasionally within the cells, were demonstrated in the infected animals. Pus from persons with gonorrhea of longer standing, with a small number of cocci, failed to produce the infection. The disease cleared up spontaneously within a few weeks; it remained localized. The complement-fixation test for gonorrhea with the blood serum of the infected animals was negative.

I. DAVIDSOHN.

Immunology

THE RELATIVE PRECIPITIN RESPONSE OF VARIOUS BREEDS OF RABBITS. J. T. CULBERTSON, Am. J. Hyg. **22**:190, 1935.

Rabbits of all the breeds tested were found capable of a powerful precipitin response on immunization with crystallized egg albumin. In one breed, however, Black Dutch, in which a considerable number of animals were used, both poor and good responses were obtained, the ratio of the two types of response being 1:41. When two animals which responded well were mated, three of their four offspring also responded well, the fourth poorly. When two which responded poorly were mated, four of five young responded poorly, the fifth well. The three young of a female with a good response mated with a male that gave a poor response all responded well. Selected rabbits of the Black Dutch breed were found to be individually consistent in the amount of antibody produced when tested repeatedly

over a period of more than a year, poorly productive animals failing to improve and abundantly productive animals maintaining their ability. Animals were found to respond satisfactorily with agglutinin to a suspension of typhoid bacilli when they failed on test before and after to produce antibody well against crystallized egg albumin.

FROM THE AUTHOR'S CONCLUSIONS.

ISO-ELECTRIC ZONES OF EBERTHELLA TYPHOSA AND BRUCELLA ABORTUS AGGLUTININS. S. R. DAMON and A. A. HAJNA, Am. J. Hyg. **22**:392, 1935.

The determination of the iso-electric zone of the typhoid agglutinating antibody by Ottenberg and Stenbuck is confirmed. The turning point of this agglutinin as determined by Damon and Hajna is between p_H 4.35 and 4.60. The iso-electric zone of Brucella abortus agglutinin in bovine serum is between p_H 5.57 and 5.65. The turning point of this agglutinin is very close to p_H 5.60.

FROM THE AUTHORS' SUMMARY.

THE HETEROPHIL ANTIBODY TEST IN LEUKEMIA AND LEUKEMOID CONDITIONS. G. L. WEINSTEIN and T. FITZ-HUGH JR., Am. J. M. Sc. **190**:106, 1935.

The titer of heterophil antibody in the serum of sixteen persons with leukemia was found to be uniformly and repeatedly at a low level (zone 1) regardless of the stage and type of the disease. In one patient with acute myelogenous leukemia it was found to be in zone 2. This patient, however, had received twenty-eight blood transfusions. This constant finding may be of value in ruling out the diagnosis of leukemia in any case in which a high titer of heterophil antibody is found. A low or normal titer of heterophil antibody was found also in three cases of Hodgkin's disease, five of lymphosarcoma, five of polycythemia vera, four of agranulocytic angina and a number of miscellaneous cases of typhoid fever, simple adenitis, syphilis, tuberculosis, anemia, etc. A high titer (zone 3) was found in cases of serum sickness and acute infectious mononucleosis, thus confirming the reports of Paul, Bunnell, Davidsohn and others. The parenteral administration of horse serum did not produce a rise in the titer of heterophil antibody in five cases of chronic lymphatic leukemia. This finding is in accord with previous evidence. A similar failure of increase in the titer of heterophil antibody following injections of horse serum was found in one case of so-called atypical Hodgkin's disease and two of lymphosarcoma, suggesting the possibility of a biologic relationship of these conditions to lymphatic leukemia. The parenteral administration of horse serum to three patients with chronic myelogenous leukemia produced a marked rise in the titer of heterophil antibody similar to that occurring in nonleukemic persons. This finding is not in accord with previous evidence and suggests the possibility of a real biologic difference between myelogenous leukemia, on the one hand, and the lymphatic group, on the other.

FROM THE AUTHORS' CONCLUSIONS.

MONOCYTES AS A SOURCE OF ALVEOLAR PHAGOCYTES. J. UNGAR JR. and G. R. Wilson, Am. J. Path. **11**:681, 1935.

A technic for preparing a suspension of pigment-marked cells in one animal and transferring it from this animal to others is described. The complemental power of serum enhances the phagocytic properties of homologous leukocytes in vivo and in vitro, increasing the number of pigment-containing cells, the number of particles per cell and the rapidity of the process. Viable phagocytic mononuclears marked by the ingestion of pigment are concentrated in the lungs regardless of the site of injection of the cell suspension. Alveolar phagocytes are derived largely, if not entirely, from monocytes of the circulating blood.

FROM THE AUTHORS' SUMMARY.

THE PHARMACOLOGIC ACTION OF TUBERCULOPROTEIN IN NORMAL AND TUBERCULOUS ANIMALS. M. I. SMITH, Am. Rev. Tuberc. **32**:98, 1935.

Tuberculoprotein has a primary toxicity in normal animals but is much more toxic in animals infected with tubercle bacilli. Tuberculoprotein injected intravenously into tuberculous rabbits under anesthesia produces a progressive decline in arterial blood pressure and a failure in the response of the vasomotor center to afferent stimuli. It is without apparent effect on the surviving blood vessels of normal or tuberculous guinea-pigs. The surviving blood vessels of the tuberculous guinea-pig in severe tuberculin shock respond normally to drugs acting on the peripheral vascular mechanism. Comparative tests of anaphylactic and tuberculin hypersensitiveness made on animals that had been simultaneously infected with tubercle bacilli and sensitized to horse serum have shown that the two phenomena are distinct and independent of each other.

H. J. CORPER.

MILK ALLERGY. B. RATNER, J. A. M. A. **105**:934, 1935.

Hypersensitiveness or allergy to milk is of more frequent occurrence than is generally realized. It not only causes mild symptoms but may even result in anaphylactic death. It occurs in adult life as well as in infancy and childhood. The soluble whey proteins of raw cow's milk, lactalbumin and lactoglobulin, are most often responsible for this condition; casein plays a negligible rôle. The pathogenesis, which is accounted for on the basis of acquisition and not of inheritance, suggests preventive measures. The treatment consists in the elimination of raw milk from the diet, replacement of it by denatured milk and the establishment of tolerance by the slow and gradual introducton of raw milk.

FROM THE AUTHOR'S SUMMARY.

A HEMOLYTIC BLOOD TRANSFUSION REACTION WITH OLIGURIA. H. G. MCCANDLESS, J. A. M. A. **105**:952, 1935.

A typical case of hemolytic blood transfusion reaction with oliguria was studied, together with twenty-eight cases that had been reported previously. The peak of the reaction after the transfusion usually occurred on the ninth day. In the twenty-nine cases there were eighteen deaths, a mortality of 62 per cent. Neither recovery nor death depended on the method of the transfusion or on the amount of blood given. The reaction appears to be one of hemolysis and cannot be forecast by cross-matching.

FROM THE AUTHOR'S SUMMARY.

IMMUNOLOGIC SPECIFICITY OF STAPHYLOCOCCI. L. A. JULIANELLE and C. W. WIEGHARD, J. Exper. Med. **62**:11, 23 and 31, 1935.

Serologic Types.—Agglutination is not a precise method for the demonstration of serologic types among staphylococci. Precipitation of soluble specific substance derived from these organisms demonstrates the existence of at least two immunologically distinct types. The one type, designated A, is composed of apparently virulent strains, while the other, type B, embraces the avirulent strains. Precipitation tests with centrifugates of young broth cultures or with acid extracts of sedimented bacteria may also demonstrate type specificity. Lysis by bacteriophage fails to detect the specific types of staphylococci. Immunization by intravenous methods stimulates formation of agglutinin in all rabbits and formation of precipitin in only one of three or four animals. Immunization by repeated intracutaneous injections of dead or of living staphylococci in an agar focus also stimulates formation of agglutinin but fails to incite formation of type-specific precipitins.

Carbohydrates.—Two immunologically and chemically distinct carbohydrates have been extracted from different strains of staphylococci. The chemical differences are manifested principally in optical rotation and in the simple sugars resulting

from hydrolytic cleavage of the specific carbohydrates. The immunologic specificity of both polysaccharides is dissipated as hydrolysis proceeds.

Relations of Cell Constituents.—The carbohydrates derived from staphylococci are type-specific. The specific carbohydrates fail to induce formation of antibodies in rabbits. Acetylation or adsorption of the carbohydrates on collodion particles does not render them antigenic. The specific carbohydrates may be employed to elicit immediate type-specific skin reactions in patients with staphylococcic infection. Staphylococcic protein is species-specific. The protein is antigenic and stimulates in rabbits species-specific antibodies. In hypersensitive persons it causes a delayed species-specific inflammatory skin reaction.

FROM THE AUTHORS' SUMMARIES.

EFFECT OF PNEUMOCOCCIC AUTOLYSATE TOXIN AND ANTITOXIN IN MICE. J. T. WELD and A. GUNTHER, J. Exper. Med. **62**:119, 1935.

Certain anaerobically produced autolysates of pneumococci injected intravenously in quantities of from 0.1 to 0.2 cc. killed mice within from a few hours to eight days. The symptoms of the mice were weakness and increasing prostration until death. Massive albuminuria appeared eighteen hours after injection of the toxin. During the course of prolonged intoxication ascites and edema of the subcutaneous tissues developed in some instances. Large pale yellow or white kidneys were found in mice that survived five days. In the latter animals, emaciation was usually marked at death. The antitoxin prepared in horses by immunization with the autolysates neutralized the autolysates whether mixed with them in vitro before injection or injected separately after the autolysates. The injection of pneumococcic autolysate toxin incompletely neutralized with the antitoxin caused a protracted intoxication with symptoms and pathologic changes similar to those found in mice dying slowly after injections of the toxic autolysate alone.

FROM THE AUTHORS' CONCLUSIONS.

STUDIES ON THE IMMUNE RESPONSE OF THE RHEUMATIC SUBJECT AND THE RELATIONSHIP OF THIS RESPONSE TO ACTIVITY OF THE RHEUMATIC PROCESS. A. F. COBURN and R. H. PAULI, J. Exper. Med. **62**:129, 127 and 159, 1935.

Antistreptolysin Titer.—A method for determining the titer of antistreptolysin is described in detail. The natural human level determined in this way is approximately 50 units.

Epidemic of Influenza Followed by a Hemolytic Streptococcic Infection in a Rheumatic Colony.—A study has been made on an isolated group of children with heart disease. All, with one exception, were subject to rheumatic attacks. Many carried a strain of hemolytic streptococci in the flora of the throat during the winter of 1934. This strain produced no detectable toxin and was not associated with respiratory disease. Four patients contracted chickenpox during the winter months. None experienced rheumatic recrudescences. All of the children were in good health on March 1. A severe epidemic of influenza began on March 22. All but six children contracted the disease. The filtrable virus responsible for this outbreak was recovered. This agent did not activate the rheumatic process. The epidemic was followed by an outbreak of streptococcic infection and appeared to facilitate the spread of the latter. The source of the streptococcic infection was not traced. It was due to a single type of hemolytic streptococci, which was a strong toxin producer. In cultural, biochemical and serologic characteristics it differed from the carrier strain. Of seventeen children proved bacteriologically to be infected with the epidemic strain, fourteen who were rheumatic subjects were seized with acute rheumatism; two rheumatic subjects and one patient with congenital heart disease escaped. The fourteen rheumatic attacks were accompanied by a rise in the titer of antistreptolysin coincident with the onset of symptoms. In four of these attacks it was possible to exclude influenza as a causative factor.

Reactions of a Rheumatic Group to an Epidemic of Infection with Hemolytic Streptococci of a Single Type.—This study of an isolated colony showed that of seven children who escaped the epidemic of streptococcic infection none had rheumatic symptoms, and that of seventeen children who contracted the streptococcic infection fourteen presented acute rheumatism and three showed no recognizable rheumatic manifestations. The seven children who failed to contract the infection with Streptococcus haemolyticus showed clearly that susceptible persons may live in close association with an epidemic of acute rheumatism, reveal no rise in the titer of antistreptolysin and maintain excellent health. The patient with congenital heart disease demonstrated that a nonrheumatic subject may be infected with a highly infective strain of hemolytic streptococci and develop a typical antibody response yet escape all rheumatic manifestations. The two patients who, though infected with the epidemic strain, failed to show any antibody response also failed to experience rheumatic recrudescences. Environment, diet, age and other factors investigated did not appear to be significant in this outbreak of acute rheumatism. Three factors appeared to determine the development of the fourteen recrudescences: (1) infection with a highly infective agent; (2) the disease pattern, peculiar to each rheumatic subject; (3) the intensity of the immune response of the patient as indicated by the rise in the titer of antistreptolysin.

From the Authors' Summaries.

The Antigenic Action of the Phosphatides (Cephalin). A. Wadsworth, E. Maltaner and F. Maltaner, J. Immunol. **28**:183, 1935.

Mixtures of horse serum albumin, cephalin and eight-hundredth normal hydrochloric acid gave a precipitate. This was inoculated into rabbits. Complement-fixation tests were carried out with the serum of the rabbits and with serums of other animals that were treated with various fractions of serum without cephalin. There was no evidence of antigenic activity in purified cephalin, and the effect of the combination was that of lowering the antigenic efficiency of the albumin fraction.

I. Davidsohn.

Nature of the Postanaphylactic State in Guinea-Pigs. I. Ostromislensky and M. Openchowski, J. Immunol. **29**:13 and 19, 1935.

When a sensitized guinea-pig receives a sublethal dose of the homologous antigen, it appears outwardly normal, but, according to Ostromislensky and Openchowski, it shows a change in reaction to subcutaneous injections of different azo compounds, of which mainly 2:4- di-amino-azobenzene was studied. If this compound is administered to the sensitized guinea-pig between the eighteenth and thirty-first day after the reinjection of the sublethal dose of antigen a characteristic reaction occurs which resembles anaphylactic shock in rabbits. When the compound is given before the eighteenth day no such reaction occurs, and the subsequent reinjection of the same chemical at a time when the reaction usually takes place does not provoke it. Ostromislensky discovered a number of antishock preparations which prevent or alleviate anaphylactic shock in animals. In the present study the same compounds were found capable of preventing the described reaction to azo compounds in the postanaphylactic state. The injection of di-phenylformamidin hydrochloride into normal guinea-pigs provoked the same symptom complex as was observed during the postanaphylactic state following the injection of 2:4- di-amino-azobenzene hydrochloride. The same reaction occurred when a solution of acetanilid was injected into sensitized guinea-pigs preceding the reinjection of the homologous antigen. Di-phenylformamidin belongs to the group of antishock preparations. It was found that a marked elevation of blood sugar followed the injection of each of these compounds.

I. Davidsohn.

ANTI-INFLUENZAL SERUM. P. P. LAIDLAW and others, Brit. J. Exper. Path. **16**:275, 1935.

Anti-influenzal serum of significant potency can be made by hyperimmunizing horses with virus-containing emulsions from ferrets suffering with acute influenzal attacks caused by human or porcine strains of influenza virus. Such hyperimmune serum, even after considerable dilution, neutralizes influenza virus. It confers some degree of passive immunity on mice following injection. It has a beneficial action on mice already infected with the virus. It can be fractionated to obtain a concentrated preparation. The greater part of the active substance is found in the pseudoglobulin fraction of the serum and is precipitated in the fraction salted out by concentrations of sodium sulfate between 12 and 16 per cent. The resolution of influenzal pneumonia in mice is a slow process and presents some peculiar features.

FROM THE AUTHORS' SUMMARY.

Tumors

ANGIOBLASTIC MENINGIOMA. H. BERGSTRAND and H. OLIVECRONA, Am. J. Cancer **24**:522, 1935.

Four so-called angioblastic meningiomas are described. It is concluded, however, that these tumors are not tumors of blood vessels but a peculiar variety of meningioma. The blood vessels are more numerous than in the ordinary meningioma but small.

INFLUENCE OF CALORIC INTAKE ON THE GROWTH OF SARCOMA 180. F. BISCHOFF, M. L. LONG and L. C. MAXWELL, Am. J. Cancer **24**:549, 1935.

A restriction of normal caloric intake by 20 per cent with and without compensation in metabolism (as judged by weight changes) produced no effect on the rate of growth of mouse sarcoma 180 in normal animals. By restricting the caloric intake 33 per cent, doubtfully significant effects were produced on tumor growth. By restricting the caloric intake 50 per cent, marked effects were produced, while the losses in body weight were barely less than those with a 33 per cent restriction. Tumors markedly retarded in growth by starvation on reinoculation grew significantly less than reinoculated controls. Thyroxin, U. S. P., in doses approximating those used by Gilroy failed to affect tumor growth. The caloric intake was increased 20 per cent, with a slight decrease in body weight. In animals in a poor nutritional state (as judged by loss in body weight) reduction in caloric intake produced a more marked effect on tumor growth than was produced in more normal animals.

FROM THE AUTHORS' SUMMARY.

THE SUSCEPTIBILITY TO CANCER DEVELOPMENT IN THE SKIN AND IN THE MAMMARY GLAND IN TWO LINES OF INBRED MICE. L. KREYBERG, Am. J. Cancer **24**:554, 1935.

Twelve hundred albino mice, including fifteen generations, all descending from one female of unknown stock, have been painted with tar under strictly uniform experimental conditions. The males have shown a distinctly delayed tar tumor reaction as compared with the females. The first thousand animals, regarded as a population, have shown a constant tar tumor reaction during a period of five years, but the distribution of tar tumors in different family lines has shown marked segregation. The segregation observed in two lines was especially marked, the one line giving a very high incidence of spontaneous cancer of the breast and the other line no cancer of the breast at all. The line with no cancer of the breast has shown, males as well as females, an earlier appearance and a higher incidence of tar cancer than the male and the female population. Vice versa, the line with a

high incidence of cancer of the breast has shown a lower incidence of tar tumors than the population. This is true also for the males, which are not subject to cancer of the breast. They have not shown any compensating increase in tar cancer.

FROM THE AUTHOR'S SUMMARY.

THE SIGNIFICANCE OF ABNORMAL MITOSIS IN THE DEVELOPMENT OF MALIGNANCY. W. MENDELSOHN, Am. J. Cancer **24**:626, 1935.

Many abnormal mitotic figures have been found in the inflammatory reaction induced by the larvae of Taenia crassicollis fifteen days after ingestion of the ova by the rat. Similar abnormalities have not been found in the inflammatory reaction aroused by the implantation of powdered mustard in the liver of the rat. The onset of malignancy in cysticercus cysts may be associated in some way with the abnormal mitoses. These seem to be a manifestation of a potential carcinogenic agent at work.

FROM THE AUTHOR'S SUMMARY.

FATTY TISSUE TUMORS OF THE BREAST. J. G. MENVILLE, Am. J. Cancer **24**:797, 1935.

It is believed that fat necrosis and xanthomatous degeneration in the breast arise from fatty tissue and should, with lipomas, be classified as fatty tissue tumors. Fatty tissue tumors of the breast are rare, 58 occurring among approximately 3,000 tumors of the breast. The underlying cause of fat necrosis and xanthomatous degeneration is believed to be a local disturbance in the metabolism of lipid produced by secondary factors such as trauma, ischemia, etc. At times fatty tissue tumors cannot be clinically differentiated from malignant growths. Biopsy and examination of frozen sections are essential in the diagnosis and treatment of these tumors.

FROM THE AUTHOR'S CONCLUSIONS.

A QUANTITATIVE STUDY OF THE GROWTH OF THE WALKER RAT TUMOR AND THE FLEXNER-JOBLING RAT CARCINOMA. R. SCHREK, Am. J. Cancer **24**:807, 1935.

The smaller the amount of inoculum of tumor cell suspension, the smaller is the percentage of takes, and the longer is the latent period; but the amount of the inoculum has little if any effect on the rate of growth of the resulting tumor.

FROM THE AUTHOR'S CONCLUSIONS.

CARCINOMA OF THE MAMMARY GLAND IN AN INBRED STOCK OF ALBINO MICE. A. C. WILLIAMS, L. E. SILCOX and B. HALPERT, Am. J. Cancer **24**:823, 1935.

Two groups of mice with spontaneous tumors belonging to the "Albino A Stock" of Strong of the forty-fifth to forty-eighth generations of brother-to-sister mating were studied. In one group the growths were relatively early, in the other, advanced. Among the 16 mice with early tumors, 13 had one and 3 had two growths. In the 74 mice with advanced tumors, 57 had one and 17 had two or three growths. The optimum age for the appearance of the growths was the eleventh month of life. In the majority of the growths the epithelial cells formed solid masses; in some, acinar or tubular structures; in others, both types were represented. Nests of epithelial cells in a concentric arrangement about areas of keratinization were observed in the growths of two mice. A stroma composed of spindle-shaped cells forming interlacing bundles was seen in the growths of 2 mice with early and in those of 7 with advanced tumors. Metastases were seen in 5 of the mice with early and in 29 of those with advanced growth. Metastases occurred in the lungs only. No correlation could be detected between the site of the primary growth and the frequency of metastases.

FROM THE AUTHORS' SUMMARY.

THE MALIGNANT TUMORS OF THE PERIPHERAL NERVES. A. P. STOUT, Am. J. Cancer **25**:1, 1935.

There are two classes of primary malignant tumors which develop in the peripheral nerves—those of mesoblastic origin and those derived from neuro-epithelium. The mesoblastic tumors form by far the largest group. They can be subdivided on histologic grounds into the uncommon malignant neurofibroma and the common fibrosarcoma. The malignant neurofibroma reproduces the simple neurofibroma on a large scale and with the development of atypical cell forms. The fibrosarcoma is made up of spindle cells, which are arranged in interlacing bundles, and of collagen fibers, which tend to be wrapped about every cell. The great majority of all these tumors occur in persons suffering from Recklinghausen's disease. The striking clinical features include persistent growth, frequency of reappearance after attempted surgical removal and metastasis in 20 per cent of the cases of fibrosarcoma. Of tumors reported as belonging to the neuro-epithelial group only three are acceptable. These presented varying histologic features. Four other recorded tumors which may have been primary malignant neuro-epithelial growths are discussed but are rejected for lack of proof. One of these was probably a metastasis from a primary tumor of the lung. The tumors derived from ganglions which happen to be situated within various nerves are not considered primary tumors of the nerves. They are referred to briefly, and one pigmented paraganglioma of the ganglion nodosum situated in the vagus nerve is reported in illustration.

FROM THE AUTHOR'S SUMMARY.

FOWL TUMORS INDUCED BY CARCINOGENIC AGENTS. P. R. PEACOCK, Am. J. Cancer **25**:37, 1935.

A seasonal variation in the rate of growth and transmissibility of induced fowl sarcoma has been noticed. This factor has been observed during two successive seasons and appears to be of a general metabolic nature, overriding considerations of the genetic relationship, age and sex of the birds and the histologic type of the tumor.

FROM THE AUTHOR'S SUMMARY.

CHORIONEPITHELIOMA IN THE MALE. H. C. FORTNER and S. E. OWEN, Am. J. Cancer **25**:89, 1935.

Tests for prolan in two cases of chorionepithelioma of the testis offered confirmation of the diagnosis. The urinary output of prolan in the presence of chorionepithelioma of the testis exceeds that associated with pregnancy and testicular tumors of the teratoma type.

FROM THE AUTHORS' SUMMARY.

MELANOMA OF THE CHOROID: THE PROGNOSTIC SIGNIFICANCE OF ARGYROPHIL FIBERS. G. R. CALLENDER and H. C. WILDER, Am. J. Cancer **25**:251, 1935.

One hundred and twenty cases of melanoma of the choroid followed for a year or longer form the basis of this report. No metastases occurred when all areas of the primary tumor contained argyrophil fibers. Metastases occurred in 36 per cent of the cases in which some areas of the primary tumor contained no fibers and in 57 per cent of the cases in which fibers were present only in the stroma of the primary tumor. The results are more striking in the cases followed five years or longer. In these no deaths occurred when fibers were present throughout all areas of the primary tumor. In the mixed group, i. e., those in which there were some areas without fibers, 68 per cent of the patients died. In the group in which no fibers were present except in the interlobular stroma, all the patients died. The average postoperative duration of life among patients dying of tumor bears out the conclusions drawn from these data, as follows: 1. Classification according to fiber content is particularly valuable for subdividing melanomas of the mixed cell type into groups of relative malignancy, and deaths in the spindle cell group appear to be explained by the presence of areas containing no argyrophil

fibers. 2. Classification according to fiber content in conjunction with cell typing affords an aid in prognosis, the production of an abundance of fibers indicating a more favorable prognosis and a decrease in the production of fibers an increase in malignancy. 3. The original classification by cell type, based on hematoxylin and eosin staining, still holds, but a more accurate prognosis is made possible by the additional classification according to fiber content.

FROM THE AUTHORS' SUMMARY.

TRAUMATIC CARCINOMA OF THE BREAST. EMMET RIXFORD, Ann. Surg. **102**:814, 1935.

Rixford states that various criteria have been put forward in connection with the rôle of trauma in producing carcinoma. These include: a definite injury of considerable severity, a development of tumor at the injured spot; in the case of the breast, a tumor which histologically is of known form of tumor of the breast; a tumor discovered three weeks or more after the accident (German practice) and not larger than a tumor would be likely to have grown in the interval following the accident, and absence of tumor at the site of injury before the accident.

A case is described which satisfied all the requirements but the last. A 17 year old young man was severely injured in the right breast. After two months a surgeon discovered a tumor about as large as half a lemon. The surgeon considered it potentially malignant and advised its removal. About four months later the entire breast was removed but was not examined pathologically. Two and one-half years later the patient reappeared with a lump in the axilla, which, when removed, was found to be the seat of a metastatic carcinoma. He died six months later.

Rixford believes that this is a true case of traumatic carcinoma of the breast and quotes Ewing, who together with his staff had studied this case ". . . we feel that there is no escaping from the conclusion, that in all probability, if not with reasonable certainty, this boy presents a genuine case of traumatic mammary cancer."

MAX LEDERER.

EPENDYMOBLASTOMA IN THE FOURTH VENTRICLE WITH NEW BONE FORMATION. ROLAND P. MACKAY, Arch. Neurol. & Psychiat. **34**:844, 1935.

In a boy, aged 4½ years, who had had intermittent vomiting for one year, headaches and signs of cerebellar involvement (ataxic gait), a tumor was found in the fourth ventricle, protruding into the foramen magnum. Part of the tumor was soft tissue and was classified by the author as ependymoblastoma; the other part was bone and was classified as osteoblastoma. Not satisfied with a classification of the tumors and anxious to explain their origin, Mackay resorts to a hypothesis. The ependymoblastoma originated from embryonic antecedents of ependymal cells; the osteoblastoma, from meningeal rests which invaded the malocluded medullary zone (dysraphia) and proliferated there, giving rise to a tumor. Dysraphia is thus, he thinks, the essential cause of the mixed tumors of the fourth ventricle in his case.

GEORGE B. HASSIN.

TUMORS OF THE CORPUS CALLOSUM. HAROLD C. VORIS and ALFRED W. ADSON, Arch. Neurol. & Psychiat. **34**:965, 1935.

The report of Voris and Adson is based on a study of thirty-eight tumors of the corpus callosum. The tumors occurred in the third and fourth decades of life and either arose primarily in some part of the corpus callosum or secondarily extended to the latter from the frontal lobe. Signs of increased intracranial pressure, convulsive attacks and some mental changes, with central involvement of the facial nerve and cerebellum, were present in half of their cases. Pathologically the tumors were all gliomas; 70 per cent of them were classified as glioblastoma multiforme.

GEORGE B. HASSIN.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

PERCIVAL BAILEY, *President*

Regular Monthly Meeting, April 13, 1936

EDWIN F. HIRSCH, *Secretary*

THE NATURE OF THE ANTIBODIES IN THE SERUM OF PATIENTS WITH INFECTIOUS MONONUCLEOSIS. I. DAVIDSOHN.

Absorptions with kidney tissues of the guinea-pig demonstrate that the heterophilic antisheep agglutinins in the blood serum of patients with infectious mononucleosis are not of the Forssman type. The agglutinins are readily and completely absorbed by ox erythrocytes and to some extent, though not so thoroughly, by kidney tissues of the horse. The results indicate the existence of a common antigenic substance in the erythrocytes of the ox and sheep and in the kidney of the horse. This antigen reacts with the heterophilic antibody in the blood serum of patients with infectious mononucleosis. The failure of the sheep cell agglutinins to be removed completely by the kidney tissues of the guinea-pig and their ready absorption by the erythrocytes of the ox offer an easy method of differentiation of this disease from serum disease, a condition in which absorption with kidney tissues of the guinea-pig removes the agglutinins for sheep erythrocytes. The same procedure makes it possible to differentiate from true infectious mononucleosis conditions which resemble it clinically and hematologically and in which the titers indicate an elevation of the level of sheep agglutinins. The technic of the differential test is discussed and its value illustrated with case reports.

DISCUSSION

E. F. HIRSCH: Do your studies lead you to conclude that infectious mononucleosis is a specific disease entity, or is the term used simply to include a group of the manifestations of disease?

R. H. JAFFÉ: I had in mind a similar question and the question whether you consider the disorder due to the action of some virus or other agent. The monocytes of the blood are really irritation forms, and the blood conditions are fundamentally different from leukemia. There are no "blast" cells in infectious mononucleosis. One should be careful as to the terminology used.

A. S. GIORDANO: These studies are interesting and demonstrate the great help which serology offers in differential diagnosis. The chronic forms of lymphatic leukemia are difficult to separate, except that in infectious mononucleosis the "blast" cells are absent.

I. DAVIDSOHN: I value the serologic tests not only in diagnosis but also in defining a group of patients whose disease is a specific entity. There is nothing so specific as the serologic response. I know of no better term than the one, "infectious mononucleosis," now in use. The cause of the disease is not known, and I have no exact opinion.

HODGKIN'S DISEASE (MALIGNANT LYMPHOGRANULOMATOSIS) AND CIRRHOSIS OF THE LIVER. PAUL E. STEINER.

A white man of 55 years had for about two years painless enlargement of the superficial lymph nodes. Biopsy of an inguinal node about three months before death revealed the histologic picture of malignant lymphogranuloma

(Hodgkin's disease). Following roentgen therapy the enlarged superficial lymph nodes disappeared, showing the characteristic therapeutic response. However, a portal obstruction developed with jaundice, ascites, rectal bleeding and anemia. Postmortem examination revealed lymphogranulomatous involvement only of the lymph nodes on the left side of the pelvis about the iliac vessels and along the left side of the abdominal aorta. Microscopically the enlarged nodes were scirrhous, only small foci of active lymphogranulomatous tissue persisting in them. This was a spontaneous fibrosis, these regions having never been irradiated. Lymphogranulomatous tissue was also present in the liver, spleen and vertebral bodies.

The liver was small, firm and finely nodular. It weighed 855 Gm. Microscopically it had a monolobular periportal cirrhosis with little active necrosis, fatty change or regeneration, although there was some distortion. In scattered periportal areas were cellular granulomatous foci with the diagnostic features of Hodgkin's lymphogranuloma. Other areas, while still typical of the same disease, showed increasing amounts of fibrosis, while other periportal foci considered to be analogous but further advanced had only fibrous connective tissue.

Just as in the lymph nodes spontaneous fibrosis occurred to an unusual degree, so in the liver the unusually widespread periportal lymphogranulomatous infiltrate underwent fibrosis. The result of this process was seen in the small, cirrhotic liver which produced the clinical effects of portal obstruction and death.

DISCUSSION

R. H. JAFFÉ: Irradiation of one group of lymph nodes is followed by a regression in other groups even though they are not in the field exposed.

CARL APPELBACH: Could there have been a chronic infection of the biliary tract with retrograde cholangeitis?

P. E. STEINER: The histologic structure of the lesions of the liver corresponds to that of the lesions in Hodgkin's disease and is unlike that of the lesions in cholangeitis.

SIGNIFICANCE OF THE TISSUE LYMPHOCYTES IN THE PROGNOSIS OF LYMPHOGRANULOMATOSIS. S. R. ROSENTHAL.

The complete report was published in the May 1936 issue of the ARCHIVES, page 628.

DISCUSSION

E. F. HIRSCH: The value of these studies in the interpretation of the disease would be considerable if we knew the exact function of the lymphocyte.

THE THROMBOTIC CONSTITUTION. WILLIAM F. PETERSEN.

A change in the wall of the vessel, in the flow of the blood current and in the constitution of the blood (plasma and formed elements) is accepted as of paramount importance in thrombosis, and attention is called to the constant variability of these factors and the dependence of this variability on the conditioning of the patient by meteorological alterations. A thrombotic accentuation is apt to follow a series of undue pressor episodes, with overstimulation (and fatigue) of the tissues. At such periods the diastolic blood pressure is unusually low, the endothelium sticky, the fibrinogen of the blood increased and the organic status relatively acid. When thrombosis occurs, the environmental situation may find clinical expression in seemingly wholly different episodes in other persons.

MALIGNANT ENDOCARDITIS CAUSED BY BACILLUS PYOCYANEUS: REPORT OF A CASE. J. J. KEARNS.

The complete report will be published in the ARCHIVES.

DISCUSSION

CARL APPELBACH: Were there old scars of the valve suggesting a previous lesion?

R. H. JAFFÉ: When a positive Widal reaction was obtained with the serum of this patient an absorption test was made. Serum of the patient treated with a suspension of typhoid bacilli still had a high agglutination titer for Bacillus pyocyaneus.

J. J. KEARNS: The myocardium had no Aschoff bodies. The high agglutination titer of the serum for the typhoid bacillus is not explained. There is a history of typhoid fever in 1918.

Book Reviews

A Textbook of Laboratory Diagnosis with Clinical Applications for Practitioners and Students. By Edwin E. Osgood, M.D. Assistant Professor of Medicine and Biochemistry and Director of Laboratories, the University of Oregon School of Medicine. Second edition. Price, $6. Pp. 585, with 27 figures, 37 tables and 10 colored plates. Philadelphia: P. Blakiston's Son & Co., 1935.

The first edition of this textbook appeared in 1931 and was reviewed in the ARCHIVES OF PATHOLOGY (**12**:863, 1931). The second edition is dedicated to the memory of the co-author of the first edition, Dr. Howard D. Haskins (late professor of biochemistry, the University of Oregon School of Medicine, Portland, Ore.), who died in the interim.

The text has been thoroughly revised. The additions include 110 pages of text, 6 figures and 4 colored plates. The urea clearance test, the insulin coefficient, the blood bromide determination, the Friedman pregnancy test, the galactose tolerance test, the test for heterophilic antibodies in infectious mononucleosis, the determination of the content of protein in the blood and in other body fluids and the forensic application of blood grouping are included for the first time. The text is enriched by the results of investigations of the author concerning, among other subjects, the normal values for plasma proteins, for the renal threshhold and for several phases of the quantitative blood picture.

It is apparent that the theoretical as well as the practical chapters have been brought up to date not only thoroughly but judiciously. It is the achievement of a successful separation of the chaff from the wheat which enabled Osgood to present in 458 pages everything that is worth while and practical in the field of clinical pathology (excepting bacteriology). Only procedures that have stood the test of time are included. The general arrangement of the first edition is retained.

The theoretical (first) part, consisting of 266 pages, includes chapters on introductory considerations; disorders of the kidney and urinary tract with especial reference to nephritis; disorders of carbohydrate, protein and fat metabolism, with especial reference to diabetes mellitus, and disturbances of the acid-base equilibrium; pregnancy and its complications; disorders of the central nervous system, with especial reference to the differential diagnosis of coma; disorders of the gastrointestinal tract; disorders of the ductless glands, with especial reference to basal metabolism in thyroid disturbances; hematology, and disorders of the respiratory and cardiovascular systems.

The technical (second) part occupies 187 pages and deals with the use and calibration of apparatus; preparation of standard solutions; chemical and microscopic examination of the urine; blood chemistry; analysis of gastric contents; analysis of duodenal contents and bile; examination of feces; determination of the basal metabolic rate; hematologic methods; simple bacteriologic and serologic technic; examination of sputum; examination of puncture fluids, and, miscellaneous methods.

The book meets the needs of the medical student as well as of the practitioner of medicine. The excellent index of diseases, which occupies 43 pages, should prove of great value to the practitioner and to the hospital intern. It will tell them which laboratory tests are of value for diagnosing any condition with which they are confronted, and if properly used it will tend to eliminate the indiscriminate and wasteful ordering of long lists of routine examinations. To the carefully selected list of publications for collateral reading an ample number of recent titles have been added. The colored plates, the credit for which goes to Miss Clarice Ashworth, are of the same excellence that prompted Dr. A. H.

Sanford, the reviewer of the first edition, to say: "As fine as any illustrations of blood cells that have appeared in American publications."

It is indeed a pleasant task to recommend the new Osgood textbook to those who make use of the clinical laboratory as well as to those who labor in it.

Modern Criminal Investigation. By Harry Söderman, D.Sc., Head of the Institute of Police Science, School of Law, University of Stockholm, Sweden, and John J. O'Connell, Deputy Chief Inspector, New York City Police Department, and Dean of the Police Academy. Price, $3. Pp. 461, with 31 plates and 79 drawings. New York: Funk & Wagnalls Company, 1936.

This book is the outgrowth of a series of lectures delivered by the senior author at the New York City Police Academy, where there is being developed a beginning in the highly specialized European police science, of which Söderman is an outstanding representative. In a brief introduction the Police Commissioner of the City of New York states: "Experience has been and will continue to be the great teacher of police technique. . . Unless the best experience we now possess is systematized and summarized it will be lost." This is a simple statement of fact with which one can agree. The exploits of Sherlock Holmes, Philo Vance and other masters of the school of fictional criminal investigation make diverting reading. But even if a metropolitan police department had such an exponent of detective wizardry, his experience would be personal experience and would be of little value to his community after he had been "bumped off" or "taken for a ride."

In their opening chapter the authors state: "Modern police science may be said to have three phases. The first phase embraces the identification of living and dead persons. The second embraces the field work carried out by specially trained detectives at the scene of the crime. The third embraces methods used in the police laboratory to examine and analyze clues and traces discovered in the course of the investigation." It is these aspects of criminal investigation that are discussed in the book, from the opening chapter on "Psychology in Detective Service" to the final chapter on "The Police Laboratory." Admittedly, this is a wide field to cover in a single volume, but the subject matter has been judiciously selected, and the volume is almost encyclopedic in its content. Chapter XV, "Stains of Blood, Semen, Etc.," may be taken as an example of the treatment of subject matter with which the medical reader is familiar. This includes not only microchemical and micromorphologic methods but the precipitin method for the differentiation of human blood and an adequate treatment of the theory, technic and significance of blood grouping.

"The work is primarily for policemen, detectives and other peace officers." If the policeman on the beat were even superficially familiar with its contents one would not think of him as a "bull" or "flatfoot" or "pavement-pounder" but as an important element in the complicated machinery of detection of crime. It is not necessary that the patrolman on the beat should have at his fingers' ends all the information contained in this volume, but if he is intelligent and ambitious he should know that there is a book to which he can refer for the information that it contains. To facilitate his use of the volume it has been prepared in textbook style, with questions at the end of each chapter covering the subject matter. There are numerous references to the literature in the form of footnotes, and at the end is an eight page bibliography, most of the references being to the foreign literature. A nine page index facilitates reference to the contents of the volume. The book will prove interesting to the nonmedical reader who may wish to know what proper criminal investigation involves. The physician who is called on occasionally to do medicolegal work will find in it much valuable information other than that relating to the performance of a medicolegal necropsy, with the technic of which he should already be familiar.

The publication of "Modern Criminal Investigation" is significant. That it was published at all would seem to indicate that scientific criminal investigation is beginning to receive in this country some of the attention that it should have received years ago.

Laboratory Methods of the United States Army. Edited by James Stevens Simmons, B.S., M.D., Ph.D., Major, Medical Corps, United States Army; Director of Laboratories, Army Medical Center; Director of the Department of Preventive Medicine, Army Medical School. Associate editor, Cleon J. Gentzkow, M.D., Ph.D., Major, Medical Corps, United States Army; Chief of the Division of Chemistry, Army Medical School. Approved by the Surgeon-General of the United States Army. Fourth edition. Price, $6.50. Pp. 1,091, with 70 illustrations and 133 tables. Philadelphia: Lea & Febiger, 1935.

The present edition is dedicated to Colonel Charles Franklin Craig, the editor-in-chief of the third edition. The 49 chapters and the supplement are the work of 21 contributors. The book is divided into 11 parts which deal with clinical pathology (235 pages), chemistry (299 pages), mycology (18 pages), bacteriology (235 pages), filtrable viruses (29 pages), protozoology (67 pages), helminthology (15 pages), entomology (27 pages), pathology (36 pages), special veterinary laboratory methods (28 pages) and statistical methods (38 pages). The technic of the Wassermann test of the Veterans' Administration is presented in a supplement of 13 pages.

The book is intended primarily for army diagnostic laboratories and for use as a teaching manual in the army medical school. The wealth and completeness of the contents, the clarity of presentation, the easily legible type, the size and the flexible cover make the book well suited for the purpose for which it is intended.

It covers a larger ground than any book of similar size on the subject of laboratory technic and includes topics that are rarely treated of in such manuals, for instance, the preparation of iodized poppy-seed oil 40 per cent and the examination of foods and beverages. The part on statistical methods, including chapters on rates and ratios, on frequency distributions, on correlation and on significant differences shows how a difficult subject can be presented clearly and briefly.

The book is not without a few shortcomings. In a work prepared by a large number of contributors some lack of uniformity is probably unavoidable. It is therefore not surprising to find 38 pages devoted to a discussion of liver function tests and only 2 pages to tests of sputum. The laboratory worker who seeks information about the sedimentation test will hardly find it in the short paragraph of 11 lines on his procedure. The paragraph dealing with the estimation of hemoglobin content is equally superficial. On the other hand, 6 methods are given for the typing of pneumococci, and the choice among them is left to the user of the book. In the paragraph on the medicolegal application of blood grouping the important relation between groups AB and O in parents and children is not considered, and there is no mention of the factors M and N. The statement that "the blood groups are not definitely established in infants until the end of the first year of life" could be misleading unless properly qualified.

Nevertheless, the defects are of minor significance and do not detract seriously from the value of the book, which can be recommended not only to those who will be obliged to use it but also to workers outside the army. The usefulness of the text is enhanced by an accurate index.

Cardiac Output and Arterial Hypertension. By Sidney A. Gladstone, M.D. Price, $1. Pp. 56, with 4 illustrations. New York: The Author, 1935.

This short monograph deals first with a critical résumé of the foreign-gas methods of determining the output of the heart in man and describes a modification of the Grollman acetylene technic which Gladstone has developed. He claims that the error due to recirculation of blood is eliminated, without sacrificing complete mixing of the gas in the lung-bag system. This is accomplished by reducing the rebreathing time to ten seconds and by providing a rapid mixing procedure requiring about four seconds. Data are presented in support of this contention. Observations made on five patients with hypertension with this method showed that the cardiac output is not increased but remains at the normal level of 4.4 liters

per minute. The last section of the monograph presents a hypothesis of the author's that hypertension, at least the renal form, is an attempt to maintain an adequate force for glomerular filtration. The elevation in pressure is produced by some pressor substance manufactured in the kidney rendered ischemic. The site of formation of this substance is the proximal tubule, the substance entering the blood through retrograde diffusion through the loop of Henle. The author bases the hypothesis entirely on evidence from the literature. He himself has done no work on the subject. The reviewer feels strongly that this is out of place. The presentation of hypotheses arrived at by young authors who have done no research on the subject is to be discouraged. It would have been much wiser had Gladstone devised further experiments to test the accuracy of his hypothesis before presenting it for publication, especially since there is no dearth of hypotheses to explain hypertension, some of which are similar to the one presented. Furthermore, a careful perusal of the evidence presented leaves one with the impression that this is selected and not complete. Were one inclined to maintain the contrary point of view, one could assemble an equally impressive if not a more impressive, array of experiments opposed to the hypothesis. It would be far better for those interested in the theory of the causation of hypertension to refer to some of the more systematic and complete reviews available on this subject.

For readers interested in methods for determining the volume of blood flow in man the first two sections will be found useful, although it is still too early to accept without further confirmation the criticisms that the author makes concerning the Grollman technic, as well as the accuracy of his own ingenious modification.

Etudes expérimentales récentes sur les maladies infectieuses. By Jean Troisier, Professeur agrégé de pathologie expérimentale et comparée à la Faculté de Médecine de Paris, médecin de l'Hôpital Beaujon. Price, 45 francs. Pp. 280, with 50 illustrations. Paris: Masson & Cie, 1935.

This book reviews a number of selected infectious diseases with especial reference to the results of recent experimental studies. The first part is devoted to the following diseases, which are grouped together because they appear to be caused by specific agents of as yet undetermined nature: fowl sarcoma (Rous), inguinal lymphogranuloma (in French, *maladie de Nicolas-Favre, lymphogranulomatose bénigne*), yellow fever, influenza and coryza ("common cold"), vaccinal encephalitis and common jaundice (*l'ictère commun*). The second part includes a discussion of spirochetosis icterohaemorrhagica and its meningeal variant, apparently a new nosologic entity, which was first recognized and described by the author and his associates (Troisier, Jean, and Boquien, Y.: La spirochétose méningée, Paris, Masson & Cie, 1933; brief notice in the ARCHIVES [**15**:609, 1933]), visceral leishmaniasis and *la fièvre boutonneuse*, a typhus-like disease so far found mainly in the Mediterranean basin, which is caused by a form of Rickettsia. The third part of the book takes up typhoid and paratyphoid, tularemia, swine erysipelas, brucelliasis of bovine origin, tetanus, and septicemia due to the gas bacillus (Clostridium Welchii) (in French, *septicémie à Bacillus perfringens*). The next part reviews leprosy and certain forms of tuberculosis. The relations of leprosy in man to rat leprosy are discussed in detail. The forms of tuberculosis considered are tuberculous infection by way of cutaneous inoculation and the septicemic form known in French as *la typho-bacillose*. The two remaining parts of the book deal briefly with agranulocytosis and with "virus nephrotropes"—infectious agents of various kinds that have a special affinity for renal tissue.

The illustrations are good, and the style is clear and concise. Many authors are cited in the text without any reference being given to their publications. Among misspelled names may be noted Brill for Bull, Hudlesen for Huddleson, MacCoy for McCoy and Wallgreen for Wallgren. As usual in French books of this general type, there is no index, only a table of contents. The book gives an instructive account of advances in the knowledge of the diseases discussed and their clinical manifestations and diagnosis, as well as their etiology.

The Adrenals. By Arthur Grollman, Ph.D., M.D., Associate Professor of Pharmacology and Experimental Therapeutics and formerly Associate Professor of Physiology, in the Medical School of the Johns Hopkins University. Cloth. Price, $5. Pp. 410, with 17 illustrations. Baltimore: Williams & Wilkins Company, 1936.

This book presents a good analysis of the voluminous literature on the adrenal glands. It begins with a succinct résumé of the historical development of the knowledge of these glands, followed by chapters on the gross and microscopic anatomy and on the androgenic tissue—a term coined "to describe that portion of the adrenal which, in certain pathological conditions, gives rise to disorders of the reproductive system" (adrenogenital syndrome). Then comes the consideration of the physiology of the medulla and of the chemistry and pharmacology of epinephrine. The next part contains thirteen chapters on the cortex and its functional relationship under normal and abnormal conditions. The great advances in the study of the cortical secretion are clearly set forth. The last part discusses Addison's disease, tumors of the adrenal gland, the adrenogenital syndrome and other abnormalities of the adrenal gland. There is a select bibliography with seven hundred and four entries from the literature since 1850, a good subject index and seventeen illustrations. The presentation is clear and orderly. The book gives a helpful summary of the knowledge of the adrenal glands. In the epilog (pages 367 and 368) Grollman dwells on the need of scientific study of the cortical secretion, its chemical nature and physiologic effects and of the clinical use of relatively pure and potent preparations free from misleading extraneous effects. He also expresses the hope that his emphasis on the tripartite nature of the adrenal glands may stimulate the study of the androgenic tissue.

Report of the Medical Research Council for the Year 1934-1935. Presented by the Lord President of the Council to Parliament by Command of His Majesty, January 1936. Price, 3 shillings. Pp. 183. London: His Majesty's Stationery Office, 1936.

The report describes in an interesting and instructive way the activities of the Medical Research Council for the year ending Sept. 30, 1935. The Council operates under a royal charter and is supported by public moneys, with augmentations from other sources. In the introduction the relations of the Council to various lines of research are reviewed under the following heads: nutrition (the application of modern knowledge); ergot in childbirth and the isolation of ergometrine; the curative agent of pernicious anemia; the prevention of childbed fever; anesthetics; the artificial cultivation of living tissues; standards of sex hormones; iodine and thyroid disease; bedbug infestation; the toxicity of industrial solvents; traveling fellowships. These subjects give a clear idea not only of various activities of the Council but also of important recent advances in research and their practical application. The report will be of interest and instruction to all who are concerned with the advancement of medical research.

Books Received

ANNUAL MEDICAL AND SANITARY REPORT OF TANGANYIKA TERRITORY FOR THE YEAR ENDED 31ST DECEMBER 1934, INCLUDING THE ANNUAL REPORT OF THE MEDICAL LABORATORY, DAR ES SALAAM. Price, 4 shillings. Pp. 102. Dar es Salaam: The Government Printer, 1936.

SÉROTHÉRAPIE ANTIPOLIOMYÉLITIQUE D'ORIGINE ANIMALE (S. A. P.): SEIZE ANNÉES D'EXPÉRIMENTATION CLINIQUE. Auguste Pettit, Professeur à l'Institut Pasteur, Membre de l'Académie de Médecine. Paper. Price, 30 francs. Pp. 270, with 9 illustrations. Paris: Masson & Cie, 1936.

THE COURSE OF THE OESOPHAGUS IN HEALTH, AND IN DISEASE OF THE HEART AND GREAT VESSELS. William Evans. Medical Research Council, Special Report Series, no. 208. Price, 2 shillings, sixpence. Paper. Pp. 93, with 66 illustrations. London: His Majesty's Stationery Office, 1936.

CHINESE MEDICAL JOURNAL SUPPLEMENT NO. 1: PATHOLOGY AND MICROBIOLOGY, BEING MAINLY PROCEEDINGS OF THE CHINESE SOCIETY OF PATHOLOGY AND MICROBIOLOGY HELD IN CANTON, NOVEMBER 5-8, 1935. Paper. Price, $2.50. Pp. 518, with 75 plates. Peiping: Chinese Medical Journal, 1936.

BACTERIAL NUTRITION: MATERIAL FOR A COMPARATIVE PHYSIOLOGY OF BACTERIA. C. J. G. Knight. Medical Research Council, Special Report Series, no. 210. Paper. Price, 3 shillings. Pp. 182. London: His Majesty's Stationery Office, 1936.

EXPERIMENTAL EPIDEMIOLOGY. M. Greenwood, A. Bradford Hill, W. W. C. Topley and J. Wilson. Medical Research Council, Special Report Series, no. 209. Paper. Price, 3 shillings, sixpence. Pp. 204. London: His Majesty's Stationery Office, 1936.

REPORT OF THE PENROSE RESEARCH LABORATORY, FORMERLY LABORATORY AND MUSEUM OF COMPARATIVE PATHOLOGY OF THE ZOOLOGICAL SOCIETY OF PHILADEPLHIA, IN CONJUNCTION WITH THE SIXTY-FOURTH ANNUAL REPORT OF THE SOCIETY, 1936. Herbert Fox, M.D. Pp. 30. Philadelphia: Zoological Society of Philadelphia, 1936.